REMARKS

Claims 1-10 are pending and have been rejected under 35 U.S.C. § 112, first and second paragraphs. Each of these rejections is addressed below.

Support for Amendments

Claim 1 has been amended to recite a method of decreasing proliferation of an abnormally proliferating cell having decreased Sal2 protein levels, a Sal2 protein of altered molecular weight, or a proliferative disease-associated alteration in a *Sal2* nucleic acid sequence relative to a normally proliferating cell. Claim 6 has similarly been amended to recite a method to decrease DNA tumor virus replication and dissemination in a cell having decreased Sal2 protein levels, a Sal2 protein of altered molecular weight, or a proliferative disease-associated alteration in a *Sal2* nucleic acid sequence relative to a non-infected cell. Each of these methods involve contacting the abnormally proliferating cell or the virus-infected cell with a *Sal2* nucleic acid having at least 90% identity to the sequence of SEQ ID NO: 2 or SEQ ID NO: 4, thus resulting in the expression of a Sal2 polypeptide having tumor suppressive or anti-viral activity, respectively. Support for these claim amendments is found, for example, at page 2, lines 22-23; page 4, lines 26; page 19, lines 8-15; page 20, lines 30-31; Figure 7; and Table 3.

New claim 12 is directed to the administration of a *Sal2* nucleic acid by intratumoral injection into the target organ or by perfusion into blood vessels supplying the target organ. Support for this new claim is found, for example, at page 23, lines 27-30 of the specification.

New claim 13 is directed to the administration of a *Sal2* nucleic acid using viral expression vectors including, for example, adenoviral, retroviral, vaccinia viral, or recombinant adeno-associated viral vectors. Support for new claim 13 is found at page 22, lines 29-30 and page 23, lines 20-21 of the specification.

New claim 14 is drawn to the administration of a *Sal2* nucleic acid under the control of a tissue-specific promoter. Support for this new claim is found at page 23, lines 12-13 of the specification.

New claim 15 is directed to a method of decreasing proliferation of an abnormally proliferating cell having a proliferative disease-associated alteration in the Sal2 nucleic acid sequence that results in the substitution of an Arg for the Gly at amino acid position 744 of SEQ ID NO:1. Support for this new claim is found at page 38, line 28 of the specification.

No new matter has been added by any of the present amendments.

Rejection under 35 U.S.C. § 112, first paragraph

Written Description

Claims 1-10 stand rejected under 35 U.S.C. § 112, first paragraph as not being supported by an adequate written description. In applying this rejection, the Examiner asserts that the specification fails to teach the identifying characteristics and the structure-function relationship common to the genus of Sal2 nucleic acids. Furthermore, with regards to recitation of the term "a proliferative disease associated alteration in a Sal2 nucleic acid" (previously in claim 3, now cancelled, and in currently amended claim 1),

the Examiner states that, aside from the disclosed genetic alteration resulting in the substitution of a serine residue for a cysteine residue at amino acid position 73 (S73C), the specification does not provide other additional alterations leading to a proliferative disease. The Examiner therefore concludes that:

> A skilled artisan would not have viewed the teachings of the specification as sufficient to show that the applicant was in possession of the claimed invention commensurate to its scope because it does not provide adequate written description for the broad class of the genus of Sal2 nucleic acid sequence or altered Sal2 that "are associated with a proliferative disease." Therefore, only SEQ ID NOs:2 or 4 encoding the described substitution of a Cys for the Ser at position 73 of SEQ ID NO:1 meets the written description of 35 U.S.C. § 112, first paragraph.

Applicants' specification clearly describes the present claimed methods. For the foregoing reasons, this rejection should be withdrawn.

As an initial matter, applicants note that claims 9 and 10 are directed to Sal2 nucleic acid sequences encoding polypeptides having a substitution of a cysteine residue for the serine residue at position 73 of SEQ ID NO: 1. Because the Examiner has acknowledged that this nucleic acid has met the written description requirements, the 35 U.S.C. § 112, first paragraph rejection as applied to claims 9-10 should be withdrawn.

As amended, claims 1 and 6 now recite contacting an abnormally proliferating cell or a virus infected cell with a Sal2 nucleic acid having at least 90 % identity to the sequence of SEQ ID NO: 2 or SEQ ID NO: 4, wherein the nucleic acid encodes a Sal2 protein having tumor suppressive or anti-viral activity, respectively. In view of these amendments, claims 1 and 6 now have clear structural and functional limitations. Thus,

with respect to the genus of Sal2 nucleic acid sequences, applicants submit that their specification provides a written description in sufficient detail to satisfy the standard set by the Federal Circuit in *Lilly,* 43 U.S.P.Q.2d 1398. In particular, this case specifically states that the written description of a genus of DNA may be achieved by a "recitation of structural features common to members of the genus." *Lilly,* 43 U.S.P.Q.2d 1398, 1406. Thus, this aspect of the rejection should now be withdrawn.

With regards to the Examiner's assertion that the specification, in providing only one nucleic acid having a S73C mutation in Sal2, fails to adequately describe the genus of Sal2 alterations that would lead to a proliferative disease, applicants respectfully disagree.

Contrary to the Examiner's finding that only one alteration is provided by the present invention, applicants submit that a representative number of species of altered Sal2 nucleic acid is, in fact, provided by the present specification. In addition to the S73C mutation, applicants note that the specification also discloses the G744R mutation (page 38, line 28), which was detected by screening of ovarian carcinoma cell lines. In the Declaration[1] of Dr. Thomas Benjamin (dated February 14, 2003) submitted herewith, Dr. Benjamin also presents data confirming the presence of this mutation in human patients having ovarian cancer. "Page 38 (line 28) of the specification also discloses of a G744R substitution in ovarian carcinoma cell lines, which we have also found in human ovarian tumor samples." Furthermore, Dr. Benjamin also states that other

[1] Applicants note that Exhibits 1 and 2 that are referred to in this Expert Declaration are n t being submitted herewith.

polymorphisms of the *Sal2* gene have been detected in ovarian cancer. Such a polymorphism is found, for example, at amino acid position 120, and is characterized by the presence of a serine (S) or a proline (P) residue.

Furthermore, in light of the present amendment, the claimed invention now encompasses specific alterations in Sal2 in an abnormally proliferating or virally infected cell. The specification provides multiple representative species of the genus of Sal2 defects correlating with an abnormal proliferation or viral infection, which include alterations in the *Sal2* nucleic acid, a reduction in Sal2 protein levels, and the presence of a Sal2 protein of altered molecular weight. Such defects are found in up to 80% of tumor patient samples and are described in detail, for example, in Figure 7 and Table 3, page 20 (lines 30 and 31) and page 38, line 3 through page 39, line 13 of the specification. Based on the specification, a strong correlation clearly exists between defects in Sal2 and abnormal proliferation in cells because Sal2 defects are clearly present in a large proportion of ovarian tumors surveyed. Thus, contrary to the assertion in the present Office Action, the description of the claimed invention in Applicants' specification does not rely simply on the disclosed genetic alteration of the Sal2 gene. Rather, the present specification describes defects at the genetic and protein level, which affect Sal2. Applicants' specification therefore provides a description of the class of Sal2 defects encompassed by the present claims in a form entirely consistent with the standard set out in *Lilly*, in which the Federal circuit held that " [a] description of a genus of cDNAs may be achieved by means of *a recitation of a representative number of cDNAs*, defined by nucleotide sequence, *falling within the scope of the genus*..." (emphasis added) *Lilly,* 43

U.S.P.Q.2d 1398, 1406. In light of the above, applicants assert that, based on applicants' discovery of Sal2 alterations, one skilled in the art reading the specification would immediately appreciate that the methods of the present invention would be particularly useful for the treatment of proliferative diseases, such as cancer.

Even if, for argument's sake, the claimed invention were exemplified by a single genetic alteration (S73C of the *Sal2* gene) as is stated by the Examiner, applicants submit that one of skill in the art reading this specification would have readily recognized that such an alteration in this gene was merely illustrative of the broader method disclosed and claimed invention. Furthermore, one skilled in the art would also understand that applicants' invention included <u>any</u> alteration in the Sal2 nucleic acid, or any alteration in the Sal2 protein which would ultimately result in a reduction in the expression of a Sal2 protein, the expression of an altered form of the protein, or any alterations in the *Sal2* nucleic acid. It is this description that clearly conveys applicants' invention to those persons of skill in the art. This description also allows the skilled worker to identify and recognize other species falling within the present claims. Clearly, based on this description, one skilled in the art would recognize that at the time of filing, applicants' invention encompassed—not a single genetic alteration of the *Sal2* gene—but any alteration either at the nucleic acid or protein level of Sal2.

Overall, applicants have provided sufficient representative species for Sal2 defects, as required by the limitations of the claimed invention. Applicants assert that one skilled in the art reading the specification would understand the strong correlation with defects in Sal2 both at the protein and genetic level in an abnormally proliferating

cell or in virally infected cell and would in turn readily recognize that such cells would clearly respond to the methods of the claimed invention. Thus, in view of applicant's specification, one skilled in the art would understand what is encompassed by the present claims. There can be no question that applicants were in possession of the claimed genus at the time their application was filed, and that one skilled in the art would recognize applicants' disclosure as a description of the invention defined by the present claims. As a result, applicants' specification clearly satisfies the written description requirement, as set forth by the case law and the M.P.E.P., and applicants request reconsideration and withdrawal on this basis for the § 112 rejection.

Enablement

Claims 1-10

Claims 1-10 stand rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. In applying this rejection, the Examiner states that because the specification fails to teach the consensus structure of the genus of Sal2 alterations associated with a proliferation disease, one of skill in the art could not practice the invention without undue experimentation.

As is discussed above, amended claims 1 and 6 now recite contacting an abnormally proliferating cell or a virally infected cell with a *Sal2* nucleic acid having at least 90% sequence identity to the sequence of SEQ ID NO: 2 or SEQ ID NO: 4 and encoding a Sal2 protein having tumor suppressive activity or anti-viral activity, respectively. Accordingly, a skilled artisan could readily produce, without undue

experimentation, the claimed nucleic acid molecule using standard techniques in molecular biology and biochemistry. For example, the specification provides extensive, enabling details at page 21, line 17 through page 24, line 9 of the specification concerning the practice of this method. Page 39, line 17 through page 40, line 2 of the specification further teaches a method to test the tumor suppressive activity of a Sal2 expression vector by measuring proliferation by BrdU incorporation. Thus, in view of the amended claims, which now recite both functional and structural limitations, applicants respectfully request that this rejection be withdrawn.

Claims 1-8

Claims 1-8 also stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement. In particular, the Examiner states:

> ...the specification, while being enabling for decreasing proliferation of an abnormally proliferating ovarian cell with a pcDNA-mSal2 *in vitro,* wherein the genome of the ovarian cell comprises a mutation S73C in *Sal2* gene, does not reasonably provide enablement for decreasing proliferation of *any* abnormally proliferating cell *in vitro* or *in vivo* in any subject with any vector, and it does not reasonably provide enablement for decreasing the replication and dissemination of any DNA tumor virus *in vitro* or *in vivo.*

Claims 1-5

In applying this rejection, the Examiner asserts that claims 1-5 being directed to the administration of a Sal2 nucleic acid sequence to an abnormally cell are not enabled due to the general state of the art of gene therapy. Citing various references including,

for example, Verma *et al.* (*Nature* (1997) 389: 239-242; hereinafter 'Verma'), Eck *et al.* (*Phar. Basis Ther.* (1995): 77-101; hereinafter 'Eck'), Miller *et al.* (*FASEB J.* (1995) 9:190-199; hereinafter 'Miller'), Deonarain *et al.* (*Expert Opin. Ther. Pat.* (1998) 8:53-69; hereinafter 'Deonarain'), and Zink *et al.* (*Gene Ther. Mol. Biol.* (2001) 6:1-24; hereinafter 'Zink') to support the unpredictability of gene therapy strategies, the Examiner concludes:

> the specification fails to teach whether transfecting any abnormal proliferating cells, regardless the status of Sal2, would induce growth suppression and/or apoptosis, the routes, timing, and the means of *in vivo* administration so that he Sal2 could sufficiently reach the cells with a Sal2 associated abnormal proliferation; whether supplementing Sal2 would change the phenotype of the abnormally proliferating cells so that the a [sic] proliferating disease could be prevented or reversed, thus fails to provide an enabling disclosure commensurate with the scope of the claims.

Applicants respectfully traverse this rejection.

As an initial matter, applicants note that claim 1, from which claims 2-5 depend, has currently been amended and is now drawn to a method of decreasing proliferation in an abnormally proliferating cell having decreased Sal2 protein levels, a Sal2 protein with an altered molecular weight, or a proliferative disease associated alteration in a Sal2 nucleic acid sequence relative to a normally proliferating cell. Thus, as amended, claim 1 and dependent claims thereof, now recite the Sal2 status of the claimed abnormally proliferating cells, thus rendering this aspect of the rejection moot.

With respect to the Examiner's assertion that the specification fails to provide teachings regarding the routes, timing, and means of *in vivo* administration, applicants respectfully disagree and assert that the specification provides extensive enabling details concerning the administration of Sal2 to an abnormally proliferating cell applicable both in *in vivo* and *in vitro* settings. Various methods of delivery of the *Sal2* nucleic acid are described in the specification and include, for example, liposome-based transfection methods (p22, line 26) and viral delivery methods using adenoviral, retroviral, or vaccinia viral vectors (p22, lines 29-30). All of these delivery methods are generally applicable to a vast spectrum of cell types, and were standard in the art at the time of filing of the application. The specification also discloses various methods that would provide effective, targeted delivery of the Sal2 nucleic acid to abnormally proliferating cells, such as cancer cells in the ovary for example. The specification teaches, for example, at page 23, lines 6, and 27-30, that the Sal2 nucleic acid can be injected intratumorally into the target organ or into the blood vessels supplying such an organ. In this regard, the specification teaches that the Sal2 nucleic acid may be injected into the portal vein to deliver the Sal2 protein-encoding nucleic acid to the liver (see page 23, lines 29-30).

With respect to achieving high levels of expression, the specification teaches that the use of adenoviruses in particular would result in both high expression of the protein product and a large number of cells being infected due to the ability of the virus to infect non-proliferating cells. Applicants' specification further notes that while Sal2 expression from a vector of the invention may be transient (approximately one week), such expression may be sufficient to remove the abnormally proliferating cells and even

advantageous in light of possible bio-safety or toxicity concerns associated with long-term expression of a Sal2 gene (p23, lines 8-10). However, the specification teaches that the expression of Sal2 may further be prolonged by using, for example, tissue-specific promoters (page 23, lines 12-13), or by employing various improved techniques that result in sustained transgene expression and reduced inflammation. Such techniques involve, for example, the use of "second generation" vectors, which harbor specific mutations in additional early adenoviral genes and "gutless"vectors in which virtually all the viral genes have been deleted (page 23, lines 14-18), or alternatively, the use of recombinant adeno-associated viruses (rAAV), derived from non-pathogenic parvoviruses, which evoke almost no cellular response and produce transgene expression lasting months in most systems. Consistent with these teachings, even the cited Verma reference states that long-term expression can also be achieved if the recombinant adenoviral vector is administered concomitantly with immunosuppressive agents (page 241), or using second-generation 'gut-less' vectors, which can escape the immune system (p 241).

Applicants further note that dosage determination could have been carried out routinely, using methods known as of the filing date. For example, the vectors used in the method may be analyzed to determine the levels of Sal2 they produce, *e.g.*, *in vivo*. Based on this characterization, one skilled in the art could routinely determine an appropriate number of DNA molecules encoding Sal2 to inject, and after injection the activity of Sal2 produced is determined using standard methods. Dosage may therefore

be adjusted by, for example, injecting more or less vector DNA, in order to reach a known, therapeutically acceptable amount.

In spite of the Applicants' teachings and the knowledge in the art at the time of filing, the Examiner cites various references to support the lack of enablement rejection. Turning first to Deronarain, the Examiner points out the inability of gene therapy strategies to achieve long term *in vivo* expression of Sal2. However, applicants note that the claims require neither "stable" nor "long-term" transgene expression. Moreover, the claims are not limited to single-dose administration. In fact, it is quite probable that repeated, even indefinite, administration of a vector encoding Sal2 could be used to prevent and treat certain cases. Accordingly, the fact that protein expression may be transient or short-term does not undermine the enablement of the claimed invention. Furthermore, applicants note that the cited Deonarain reference does not in any way support the present rejection. Deonarain discusses various techniques in the art for gene therapy, focusing mainly on the use of ligand-targeted receptor-mediated vectors. Although the Examiner refers to this reference to show that these techniques "are even less efficient than viral gene delivery," applicants note that this reference *does not* teach that gene therapy is generally inefficient, but instead teaches that some methods are more efficient than others. With respect to ligand-targeted receptor-mediated vectors, page 65, for example, states "presently, this approach to gene delivery is much less efficient than viral gene delivery. However, *under optimal conditions, enough gene product may be produced to give a therapeutic benefit (e.g., suppress a phenotype or destroy a tumour)*" (emphasis added). Deonarain further states on page 66 "..in order to achieve the levels of

gene transfection and expression seen with retroviral vectors, further advances need to be made in the fields such as mammalian artificial chromosomes. Potentially, once genes are specifically delivered, they may be maintained for long periods of time in a way analogous to bacterial plasmids or artificial chromosomes (BACs) or yeast artificial chromosomes (YACs)." Accordingly, Deonarain does not teach the inefficiency of gene therapy and if anything, Deonarain teaches that even gene therapy strategies using the less than optimal methods (i.e. ligand-targeted receptor mediated vectors) can still achieve a therapeutic effect.

The Examiner also cites Verma to emphasize the lack of success of gene therapy given that "out of the more than 200 clinical trials currently underway, no single outcome can be pointed to as a success story." Citing Eck, Miller, and Zinck, the Examiner explains this lack of success on the various obstacles faced by gene therapy strategies, including the choice of DNA vector and the intricate regulation of gene expression in cells. To exemplify the alleged lack of success of gene therapy strategies, the Examiner refers to a study performed by Vinyals *et al.* (*Gene Ther.* (1999) 6:22-33; hereinafter 'Vinyals'), in which wild type p53 was supplemented into human cancer cells bearing a p53 mutation. The Examiner further cites U.S.P.N. 6,033,857 to attribute the lack of success of gene therapy to the inability of a single gene to participate in the development of all, or even the majority of human cancers. Turning first to the Vinyals reference, applicants note that the reference only teaches administration of p53 to human cancer cells bearing p53 mutation in cultured cell lines namely, MDA-MD468 breast cancer cells and the KM12SM colorectal cancer cells. Although cell lines expressing the

transgene following transfection were implanted into mice showed little or no tumor latency, mice having established tumors were never administered a p53-expressing adenovirus, as presently claimed. Absent of such data, this reference does not support the Examiner's position that the practice of gene therapy is not enabled. Furthermore, with regards to the Examiner's position of the lack of success of gene therapy strategies, Applicants respectfully disagree. Applicants note, for example, that a number of studies using gene therapy strategies, including those taught in the claimed invention, had been performed successfully at the time of filing of the application. Applicants further submit that such studies were even performed with p53. Applicants note, for example, that Schuler *et al.* (*Hum. Gene. Ther.* (1998) 9:207 5-2082; hereinafter "Schuler") (EXHIBIT 8) had successfully demonstrated the efficacy of gene therapy strategies in a phase I clinical trial. In this study, fifteen patients having incurable non-small cell lung cancer were administered with a single intratumoral injection of a replication-defective adenoviral expression vector encoding wild-type 53. Contrary to the Examiner's assertion, successful transfer of wild type p53 was achieved with high vector doses in the absence of significant toxicity. Furthermore, transient local disease control by a single intratumoral injection of the vector solution was observed in several patients and in one patient, a partial remission was even reported two months following injection. Similarly, Swisher *et al* (*J. Natl. Cancer Inst.* (1999) 91:763-771; hereinafter 'Swisher') (EXHIBIT 9) and Clayman *et al.* (*Clin. Cancer Res.* (1999) 5:1715-1722; hereinafter 'Clayman') (EXHIBIT 10) have also reported anti-tumor responses using similar strategies in patients having for non-small-cell lung cancer and squamous cell carcinoma of the head and neck,

respectively. The Swisher reference, for example, discloses that repeated intratumoral injections of Ad-p53 in non-small cell lung cancer patients, who could no longer respond to conventional strategies, could achieve significant transgene expression of wild-type p53 and sustained antitumor responses. In addition, Kirn *et al.* (*Proc. Am. Soc. Clin. Oncol.* (1998) 17:1509) (EXHIBIT 11) have further reported that the administration of the ONIX-015 adenovirus, which lyses p53-deficient cells, in patients having head and neck tumors, resulted in a significant portion of the tumor undergoing massive destruction, while the host tissue remained undamaged. In view of these studies, applicants submit that, at the time of filing, a person of ordinary skill in the art could have practiced the claimed invention based on the present teachings and standard molecular biology techniques without undue experimentation. In the declaration of Dr. Thomas Benjamin submitted herewith (dated July 17, 2003), Dr. Benjamin provides extensive data supporting the desirability of restoring $p150^{Sal2}$ expression in abnormally proliferating cells, such as tumor cells. Dr. Benjamin first demonstrates the strong correlation between loss of $p150^{Sal2}$ expression and the incidence of cancer (see, for example, EXHIBIT 1A-1C). Furthermore, Dr. Benjamin provides extensive data supporting the desirability of restoring the expression of $p150^{Sal2}$ as a means to reduce replication and viability of tumor cells (see, for example, EXHIBITS 2A-2I, 3A-3D, 4A-4D, 5, and 6). For example, the reinstatement of $p150^{Sal2}$ in human ovarian tumor cells induced a marked reduction in proliferation (EXHIBIT 2A), a significant increase in apoptosis (EXHIBIT 2B), suppression in colony formation (EXHIBIT 2C), and a reduction in tumorgenicity relative to tumor cells transfected with an empty vector

(EXHIBIT 2F and 2I). Conversely, DNA synthesis was significantly increased in normal human ovarian surface epithelium (HOSE) cells in which the expression of $p150^{Sal2}$ had been repressed by RNA interference (EXHIBIT 2D). Dr. Benjamin further states:

> The teachings disclosed herein are highly indicative that restoration of $p150^{Sal2}$ expression in tumor cells having a defect in Sal2 expression at the protein or RNA level, such as human ovarian tumor cells, would result in an increase in tumor cell apoptosis, a decrease in tumor cell proliferation, or both such that tumor growth would be prevented, reduced, or treated. Tumor cells that would particularly benefit from this treatment strategy include any cell that has a proliferative-associated alteration in the *Sal2* gene (e.g., S73C), reduced $p150^{Sal2}$ levels, or a $p150^{Sal2}$ protein of altered molecular weight.

In light of the above-cited examples in which gene therapy strategies were successful, applicants assert that in applying this rejection, the Examiner has erroneously equated the *inefficiency* of transgene expression with *unpredictability*. The Examiner asserts that gene therapy *in vivo* is unpredictable and is dependent upon, for example, "the fate of the vector itself (volume of distribution, rate of clearance into the tissues, etc.), the *in vivo* consequences of altered gene expression and protein function, the fraction of vector taken up by the target population, the trafficking of the genetic material within cellular organelles, the rate of degradation of the DNA, the level of mRNA produced, the stability of mRNA produced, the amount and stability of the protein produced, and the protein's compartmentalization within the cell, or its secretory fate, once produced." The test of unpredictability is not based solely on the probability of a successful outcome; even the most well-understood and well-controlled manufacturing

processes create defective products. In the art of gene therapy, a practitioner may have to create many vectors before arriving at one with successful transgene expression and desirable characteristics. None of the previously discussed references suggest that this process of making and administering such vectors to animals is anything more than *routine*. The Examiner-cited art merely identifies possible factors that contribute to the low efficiency of the process. However, case law is clear with regard to experimentation needed to practice an invention; if the experimentation necessary to practice an invention is routine, it is not a bar to patentability, even if the experimentation is not foolproof (see e.g., *Johns Hopkins University v. Cellpro, Inc.*, 152 F.3d 1342, 47 USPQ2d 1705 (Fed. Cir. 1998), and *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 231 USPQ 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987)). Thus, the Examiner, in requiring that every conceivable embodiment falling within the claims perform successfully with failures in thought experiments negating enablement, has applied a standard of perfection with respect to enablement that finds no basis in the statute or the case law. If this were the standard for enablement, generic claims would never be allowable, in any instance in which an Examiner can imagine a single inoperative embodiment. This is not the standard the law requires. For example, in *Application of Angstadt,* 537 F.2d 498, 10 U.S.P.Q. 218 (C.C.P.A. 1796), the Court, in holding that a claimed invention was enabled, even though the claims were admittedly included inoperative embodiments, stated that "the evidence as a whole, including the inoperative as well as operative example, negates the PTO position that persons of ordinary skill in this art, given its unpredictability, must engage in undue experimentation to determine which complexes

work." Therefore, applicants submit that the information provided in the specification, coupled with the knowledge provided in the art at the time the invention was made, would allow one skilled in the art to confidently and predictably practice the full scope of the claimed invention.

For all the above reasons, Applicants request reconsideration and withdrawal of the § 112, first paragraph, rejection.

Claims 6-8

The Examiner further asserts that claims 6-8 are not enabled because the specification does not teach how administering a Sal2 nucleic acid sequence is related to the replication and dissemination of any DNA tumor virus. However, applicants note that the Examiner has failed to provide support for this rejection. Applicants note that page 3, lines 7-13 of the specification teaches "a method of decreasing DNA tumor virus replication and dissemination. This method involves the step of contacting a cell infected with a DNA tumor virus, for example, a simian virus 40, human polyoma virus, herpes virus, primate adenoviruses, parvovirus, or a papilloma virus, with a *Sal2* nucleic acid sequence, where this contacting results in the expression of a Sal2 polypeptide in the cell infected with the DNA tumor virus and prevents the DNA tumor virus from replication and disseminating" (also see, for example, page 21, line 17 through page 22, line 20). Based on the results shown in EXHIBIT 7, Dr. Benjamin, in the Expert Declaration submitted herewith, further states that "a reduction in DNA tumor virus replication and dissemination could be achieved by the administration of $p150^{Sal2}$ to cells infected with a

DNA virus." Because applicants have clearly provided teachings to enable the claimed invention, the burden is on the Examiner to provide evidence of the contrary. The M.P.E.P. clearly states in the Guidelines for the Examination of Patent Applications under 35 U.S.C. § 112, first paragraph, "Enablement" requirement:

> A specification disclosure which contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject manner sought to be patented must be taken as being in compliance with the enablement requirement of 35 U.S.C. § 112, first paragraph, unless there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. Assuming that sufficient reason for such doubt exists, a rejection for failure to teach how to make and/or use will be proper on that basis. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). As stated by the court, "it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure." 439 F.2d at 224, 169 USPQ at 370.

Thus, claims 6-8 are enabled by the specification and applicants hereby request that this aspect of the rejection be withdrawn.

In sum, in view of the present amendments and remarks, applicants respectfully assert that claims 1-10 are fully enabled by the specification and request that the rejection under § 112, first paragraph for lack of enablement be withdrawn

Rejection under 35 U.S.C. § 112, second paragraph

Claims 1-8 are rejected under 35 U.S.C. §112, second paragraph for indefiniteness. In particular, the Examiner asserts that these claims fail to set forth any active and positive step and states:

> The method of claims 1 or 6 calls for contacting abnormally proliferating cells or a cell infected with a DNA tumor virus with a Sal2 nucleic acids [*sic*], however there is no positive step to recite how said contacting could be done specifically in the target cells in an *in vivo* situation.

Applicants respectfully traverse this rejection. In this regard, applicants note that based on the specification and the general knowledge in the art at the time of filing, one of ordinary skill in the art would clearly understand how contacting could be performed both *in vitro* and *in vivo.* Page 22, line 24 through page 23, line 30 of the specification teach, for example, that the Sal2 nucleic acids can be delivered by liposome-based transfection or by means of viral expression vectors. If administered in an *in vivo* setting, the Sal2 nucleic acids can be delivered either by intratumoral injection or by injection into blood vessels supplying the target organ (see page 23, lines 27-30). Applicants note, for example, that the Schuler reference teaches the administration of an adenoviral expression vector encoding p53 by bronchoscopic intratumoral injection and by CT-guided percutaneous intratumoral injection. Given these teachings, applicants respectfully request that this rejection be withdrawn.

Claim 1 is further rejected because there is no recitation in the body of the claim that clearly relates back to the preamble. This rejection has been met by the present amendment of claim 1, and may therefore be withdrawn.

CONCLUSION

Applicants submit that the claims are now in condition for allowance and such action is respectfully requested.

Applicants note for the record that the Form PTO-1449 that was submitted with an Information Disclosure Statement filed on February 20, 2003 has not been initialed and returned, and hereby request that it be initialed and returned with the next Office action.

Enclosed is a Petition to extend the period for replying to the Office action for three months, to and including July 29, 2003, and a check in payment of the required extension fee.

If there are any additional charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: July 29, 2003

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Exhibit 8

HUMAN GENE THERAPY 9:2075–2082 (September 20, 1998)
Mary Ann Liebert, Inc.

A Phase I Study of Adenovirus-Mediated Wild-Type *p53* Gene Transfer in Patients with Advanced Non-Small Cell Lung Cancer

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ABSTRACT

Mutations of the tumor suppressor gene *p53* are the most common genetic alterations observed in human cancer. Loss of wild-type *p53* function impairs cell cycle arrest as well as repair mechanisms involved in response to DNA damage. Further, apoptotic pathways as induced by radio- or chemotherapy are also abrogated. Gene transfer of wild-type *p53* was shown to reverse these deficiencies and to induce apoptosis *in vitro* and in preclinical *in vivo* tumor models. A phase I dose escalation study of a single intratumoral injection of a replication-defective adenoviral expression vector encoding wild-type *p53* was carried out in patients with incurable non-small cell lung cancer. All patients enrolled had p53 protein overexpression as a marker of mutant p53 status in pretreatment tumor biopsies. Treatment was performed either by bronchoscopic intratumoral injection or by CT-guided percutaneous intratumoral injection of the vector solution. Fifteen patients were enrolled in two centers, and were treated at four different dose levels ranging from 10^7 to 10^{10} PFU (7.5×10^9 to 7.5×10^{12} particles). No clinically significant toxicity was observed. Successful transfer of wild-type *p53* was achieved only with higher vector doses. Vector-specific wild-type *p53* RNA sequences could be demonstrated in posttreatment biopsies of six patients. Transient local disease control by a single intratumoral injection of the vector solution was observed in four of those six successfully transduced patients. There was no evidence of clinical responses at untreated tumor sites. Wild-type *p53* gene therapy by intratumoral injection of a replication-defective adenoviral expression vector is safe, feasible, and biologically effective in patients with advanced non-small cell lung cancer.

OVERVIEW SUMMARY

Gene transfer of the human wild-type (wt) *p53* tumor suppressor gene into *p53* mutant tumors has been shown to have antiproliferative and apoptotic effects *in vitro* and *in vivo*. Schuler *et al.* report on a phase I clinical study of local wt *p53* gene therapy in patients with advanced non-small cell lung cancer. A recombinant replication-deficient adenovirus carrying the complete human wt *p53* cDNA under the control of the CMV immediate/early gene promoter was devised as expression vector. Gene transfer was achieved at higher virus doses without occurrence of any relevant treatment-related toxicity. In addition, clinical effects suggestive of a moderate local antiproliferative activity were observed in some patients.

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INTRODUCTION

Mutations of the tumor suppressor gene *p53* are detected in more than 50% of human cancers. Missense mutations within the sequence coding for the DNA-binding domain of the *p53* gene product are the most common alterations observed (Hollstein *et al.*, 1994). These mutations result in synthesis of a functionally inactive protein, which accumulates intracellularly at high levels (Bartek *et al.*, 1991). Less frequently, *p53* deletions or chain-termination mutations are detected. Loss of functional p53 results in impaired cell cycle control and repair mechanisms in response to DNA damage (Levine, 1997). Patients suffering from the Li-Fraumeni syndrome are, as a result of germline mutation of the *p53* gene, prone to early cancer development, as are mice with a homozygous deletion of *p53* (Donehower *et al.*, 1992). Moreover, *p53* mutations may abrogate apoptosis as induced by several cytotoxic agents or radiation (Lowe *et al.*, 1993a,b). In light of this preclinical evidence, restoration of functional p53 represents an attractive target for somatic gene therapy in cancer. Results of a pilot study of nine patients support this view (Roth *et al.*, 1996).

Non-small cell lung cancer (NSCLC) accounts for 75 to 80% of all lung cancers, and is still a leading cause of death from malignancy (Boring *et al.*, 1994). At primary diagnosis, most patients present with nonresectable disease. Despite intensive chemo- and radiotherapy, 5-year survival of stage III patients does not exceed 15% (Dillman *et al.*, 1996; Pritchard and Anthony, 1996). In stage IV NSCLC, chemotherapy never is curative and prolongs median survival for less than 4 months as compared with best supportive care (Cellerino *et al.*, 1991; Marino *et al.*, 1994). Thus, new treatments for advanced NSCLC are clearly needed.

Structural alterations of the *p53* gene are detected in about 45 to 50% of tumors of NSCLC patients (Takahashi 1989), and are associated with an adverse prognosis (Quinlan *et al.*, 1992; Nishio *et al.*, 1996; Ohsaki *et al.*, 1996). Further, mutant *p53* status might also contribute to the low response rates to chemo- and radiotherapy observed in NSCLC. The present phase I study was undertaken to assess safety, feasibility, and biological activity in terms of transgene expression status of local adenovirus-mediated *p53* gene therapy in patients with advanced NSCLC exhibiting *p53* mutations.

PATIENTS AND METHODS

Patients

All patients enrolled in the study had histologically confirmed stage III B or IV NSCLC with evidence of *p53* gene mutation in the tumor tissue. Immunohistochemical detection of intratumoral p53 protein accumulation by monoclonal antibodies PAb 1801 and/or PAb 240 (Pharmingen, San Diego, CA) served as surrogate marker of mutant *p53* status (Bartek *et al.*, 1991). Staining of paraffin-embedded tissue was performed according to standard methods. Sections with definitive nucl ar reactivity f >50% of cells were scored 3+; sections with a reactivity of 25 to 50% of cells were scored 2+; and sections with a reactivity of 11 to 24% of cells were scored 1+. Sections with 10% or fewer reactive cells were sc red as negative. Additional inclusion criteria were as follows: age, 18 to 75 years; a Karn fsky performance score f at least 70%; an absence of clinically relevant hematologic, hepatic, or renal insufficiency or lectrolyte imbalances; and pulmonary function adequate to perform all treatment procedures safely. A treatment-free interval of at least 4 weeks in duration had to have elapsed before enrollment in the study. Pregnant or lactating women, fertile women not practicing medically acceptable contraception for at least 6 months following study treatment, patients with uncontrolled serious infections, human immunodeficiency virus (HIV) positivity, or patients receiving systemic immunosuppressive or corticosteroid treatment within the last 3 months before entry into the study were excluded. Acute adenoviral infection was ruled out prior to therapy. All patients gave written informed consent.

Methods

Study design. This was a bicentric, open, phase I dose escalation study. Three patients were treated at each dose level, and dose escalation was continued until two consecutive dose levels confirmed biological activity of the treatment as defined by demonstration of intratumoral transgene expression, or until significant dose-limiting toxicity was encountered. Each patient was monitored weekly for 28 days, with day 1 being the date of treatment. On completion of the observation period all patients were monitored at the study centers at regular intervals. The protocol was approved by the local ethics committees, and by the national regulatory offices (Germany: Kommission Somatische Gentherapie der Bundesärztekammer, and Zentralkommission Biologische Sicherheit; Switzerland: Schweizerische Kommission für Biologische Sicherheit, and Interkantonale Arzneimittelstelle). The study was performed according to the Declaration of Helsinki and according to the principles of good clinical practice.

Study end points. Primary objectives of the study were to determine safety, feasibility, and biological activity of a single intratumoral injection of SCH 58500, as defined by reverse transcription and polymerase chain reaction (RT-PCR) detection of vector-specific wild-type (wt) *p53* RNA sequences in posttreatment tumor biopsies. The secondary objective was to assess clinical evidence of antitumoral efficacy of intratumoral SCH 58500 injection in patients with NSCLC.

Staging procedures. At baseline and on day 28 all patients underwent computed tomography (CT) scans of the chest for bidimensional measurement of the treated tumor lesion and all other tumor manifestations. If clinically indicated, CT or ultrasound scans of the abdomen and bone scans were performed.

Treatment. SCH 58500 (rAd/p53) is an aqueous solution of a replication-defective recombinant adenovirus type 5 containing the complete human wt *p53* cDNA under the control of the human cytomegalovirus (CMV) immediate-early gene promotor (Wills *et al.*, 1994; Harris *et al.*, 1996). SCH 58500 was manufactured in CGMP compliance in validated plant facilities under strict environmental monitoring and control conditions by Schering-Plough Werthenstein Chemie AG (Schachen, Switzerland). In brief, the vector was propagated in 293 cells

actor. The cells were grown in liquid medium on the of microcarriers. As a part of routine testing, each fermentation batch was tested for sterility, mycoplasma, adventitious viruses, and adeno-associated virus at the unprocessed bulk stage. The SCH 58500 product was purified by chromatography (Huyghe et al., 1995) and filtration, and was supplied in vial strengths of 1×10^8 plaque-forming units per milliliter (PFU/ml) and 1×10^9 PFU/ml (7.5×10^{10} and 7.5×10^{11} particles/ml). Patients were treated with a single intratumoral injection of four different dose levels of SCH 58500 (absolute 1×10^7, 1×10^8, 1×10^9, and 1×10^{10} PFU). In dose levels 1, 2, and 3 (endobronchial) a volume of 1 ml of SCH 58500 was injected intratumorally at bronchoscopy. In dose levels 3 (percutaneous) and 4 a volume of 10 ml of study medication was administered intratumorally by percutaneous injection under CT guidance. After treatment all patients were hospitalized in single rooms in an S2 isolation unit for at least 72 hr or until detection of adenovirus shedding by the patients became negative. All patients underwent a biopsy of the treated tumor lesion 24 to 48 hr after injection of SCH 58500. In dose levels, 1, 2, and 3 (endobronchial) posttreatment biopsies were obtained by bronchoscopy. In dose levels 3 (percutaneous) and 4 posttreatment biopsies were obtained by percutaneous needle biopsy under CT guidance.

Detection of wild-type p53 gene transfer. Expression of vector-specific wt *p53* RNA was assessed in posttreatment biopsies by means of RT-PCR according to previously published methods (Wills *et al.*, 1994). In brief, total cellular RNA was isolated from homogenized tumor biopsies and reverse transcribed into cDNA. The maximum amount of RNA available was used for RT-PCR. This ranged from 0.5 to 5 μg for samples from all patients except patient UPN 015, from whom 15 μg of RNA were obtained. PCR was performed for 28 cycles for all patient samples. Samples from patients treated at low dose levels with negative PCR results after 28 cycles were also run for 45 cycles in order to increase sensitivity. Those samples were from patients UPN 001, 002, 003, 004, 006, and 007, all of which remained negative after 45 PCR cycles. The vector-encoded *p53* cDNA was amplified using specific primers, one within the Ad2 tripartite leader (at the 5′ end of the mRNA), and the second within the *p53* coding region, leading to amplification of a 563-bp target sequence. Parallel PCR reactions using primers specific for a 719-bp fragment of the human β-actin cDNA served as controls for integrity and efficacy of the RNA extraction as well as of the cDNA synthesis. Semiquantitation was carried out for vector-encoded *p53* cDNA as well as for human β-actin through the use of DNA "mimics" specific for each reaction. The mimic carried sequences at its ends that allowed it to be amplified by the same primers used to amplify the target sequence. This amplification results in a second band 412 bp in size for the "*p53* mimic," and 494 bp in size for the "β-actin mimic." Thus, on the gel, each lane should contain two bands: the upper band is the larger fragment target, and the lower band is the smaller fragment mimic. Addition of a known concentration of mimic to a given reaction enabled determination of the relative amount of each cDNA present. All PCR products and controls were run on agarose gels and stained with ethidium bromide. Quantification of the amplified DNA bands was performed on a Molecular Dynamics FluorImager (Molecular Dynamics, Sunnyval , CA).

Virology studies. Adenovirus shedding and excretion were assessed in patient stool or rectal swab by means of a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Adenoclone; Cambridge Biotech, Worcester, MA) according to the manufacturer instructions. Further, adenovirus shedding in sputum and urine was assessed by the same ELISA kit according to an adapted protocol. In addition, an ELISA system was established to detect serum antibodies against SCH 58500, which also detects cross-reacting antibodies against wild-type adenoviruses. In brief, microtiter plates were coated overnight with SCH 58500, and then were washed and blocked for nonspecific binding with bovine serum albumin (BSA). Each serum sample was diluted 1:40 into phosphate-buffered saline (PBS) containing 1% BSA and 0.05% Tween, and then was serially diluted twofold. A total of seven dilutions of each serum sample was added to the plate and were incubated overnight. A positive control and pooled normal serum were also assayed on each plate. After washing, anti-SCH 58500 antibodies were detected by reaction with biotin-labeled protein A/G (Jackson ImmunoResearch Laboratories, West Grove, PA), horseradish peroxidase-labeled streptavidin, which resulted in a colorimetric reaction on the addition of hydrogen peroxide and tetramethyl benzidine. Samples were considered positive for the presence of anti-SCH 58500 antibodies if the ratio of the mean optical densities of the sample dilutions versus the mean optical densities of the normal human serum dilutions was above a threshold value of 0.28. Further, patients were considered positive for development of anti-SCH 58500 antibodies if the posttreatment sample was positive, and there was at least a twofold increase in mean optical density as compared with pretreatment values.

RESULTS

Wild-type p53 gene transfer

Fifteen patients were enrolled in the study between October 1, 1996, and July 16, 1997. Baseline characteristics are shown in Table 1. Nine patients were treated by bronchoscopic intratumoral injection of 1 ml of SCH 58500 at three different dose levels (levels 1, 2, and 3). Six patients were treated by percutaneous intratumoral injection of 10 ml of SCH 58500 at two different dose levels (levels 3 and 4). In 13 patients sufficient amounts of intact RNA were recovered from posttreatment biopsies for analysis of transgene expression by RT-PCR. The trial was closed on August 12, 1997. At dose level 1, no transgene expression could be detected, whereas at the subsequent dose levels expression of vector-specific wt *p53* RNA sequences (Fig. 1) could be demonstrated in a way suggestive of dose dependency. Results of wt *p53* RT-PCR in relation to SCH 58500 dose levels are summarized in Table 2. The median expression level of transgenic wt *p53* amounted to 8.01 molecules per 1000 molecules of β-actin (range, 0.49 to 197.97 molecules/1000 molecules β-actin); no correlation between transgene expression level and the vector dose injected could be established.

TABLE 1. PATIENT CHARACTERISTICS

Parameter	*Value/distribution*
n	15
Sex	4 female, 11 male
Age (median, range)	60 (45 to 75) years
Histology	
Adenocarcinoma	8
Squamous cell carcinoma	4
Large cell carcinoma	3
Pretreatment	
None/local procedures	6
Surgery	4
Radiotherapy	4
Chemotherapy	9
Active smokers	14

TABLE 2. SCH 58500 GENE TRANSFER BY DOSE

Dose level (PFU)	*Patients*	*Route*	*β-Actin*[a]	*p53*[b]
10^7	3	B[c]	3	0
10^8	3	B	3	1
10^9	3	B	2	2
	3	C[d]	3	2
10^{10}[e]	3 (+1)	C	2 (+1)	1 (+1)

[a]β-Actin, RT-PCR detection of human β-actin RNA as internal control.

[b]p53, RT-PCR detection of vector-specific wt *p53* sequences.

[c]B, Bronchoscopic intratumoral injection of 1 ml of SCH 58500 solution.

[d]C, CT-guided percutaneous intratumoral injection of 10 ml of SCH 58500 solution.

[e]At dose level 4, results of the second treatment of a patient initially treated at dose level 3 (percutaneous) are included in parentheses.

Clinical response and patient follow-up

Results of day 28 restaging CT scans in relation to wt *p53* gene transfer are summarized in Table 3. Whereas local disease stabilizations have been observed at treated tumor sites, in all but one evaluable patient distant tumor sites were progressive. According to standard oncological response criteria, at day 28 restaging 11 patients had progressive disease, 2 patients had stable disease, and 2 patients were not evaluable for tumor response. In one heavily pretreated patient, who was initially enrolled at dose level 3 (percutaneous), a stabilization of the treated chest wall metastasis was observed on day 28, whereas his untreated primary tumor progressed. Approximately 4 months after the first treatment, this patient received a second dose of SCH 58500 at dose level 4 on a single patient protocol exception after approval by the local ethics committee. The second injection was directed to the patient's primary tumor. Again, successful gene transfer was confirmed by RT-PCR from a biopsy taken 24 hr posttreatment (data included in Table 2). Approximately 8 hr posttreatment the patient experienced WHO grade 2 fever. No further adverse events were observed. On restaging on day 28 after the second dose, the primary tumor was again slightly progressive. On long-term follow-up, 9 of 15 patients died from progressive disease. Another patient died on day 18 of the observation period from progressive disease. As of December 1997, three patients are still alive with clinically stable disease, one patient is alive with slowly progressing disease, and one patient has been lost to follow-up. Clinical results of the six patients with successful demonstration of transgene expression in relation to SCH 58500 dose level are shown in Table 4.

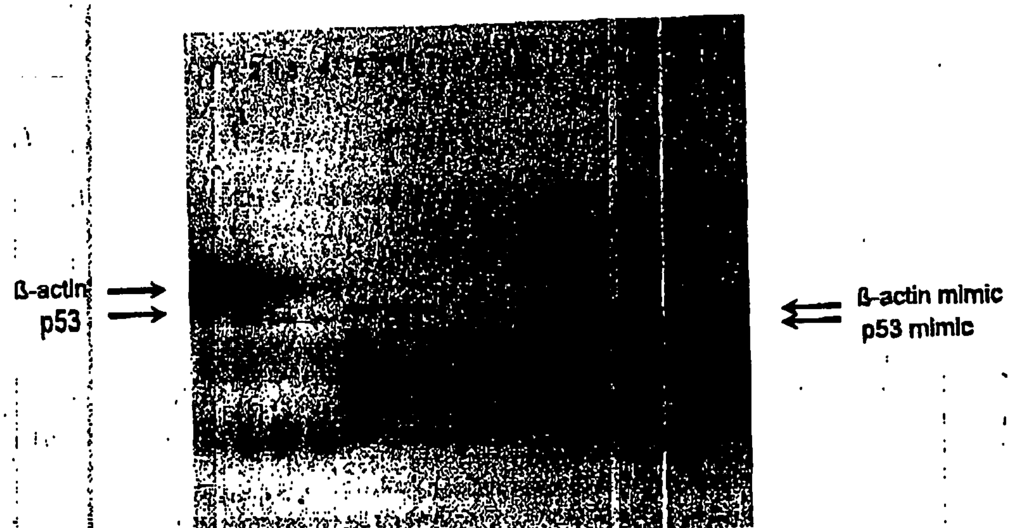

FIG. 1. Representative gel of an RT-PCR analysis of a patient sample. Four dilutions of the β-actin PCR product are shown in lanes 1–4, and four dilutions of the vector-specific *p53* PCR product are shown in lanes 5–8. A control to detect residual DNA is seen in lane 9, and DNA molecular weight marker is seen in lane 10. Low (500 molecules) and high concentrations (100,000 molecules) of the "mimics" for β-actin and vector-specific *p53* are seen in lanes 11 to 14.

TABLE 3. DISEASE STATUS ON DAY 28 IN RELATION TO WILD-TYPE *p53* TRANSGENE EXPRESSION

		Treated lesion			*Distant lesions*		
n	p53[a]	*PR*	*SD*	*PD*	*PR*	*SD*	*PD*
6	+	0	4	2	0	0	6
7[b]	–	0	2	3	0	1	3
2	ND	0	1	1	0	0	2

[a]p53, RT-PCR detection of vector-specific wt *p53* sequences.
[b]In two patients no day 28 CT scans were performed; in one patient no measurable distant disease was present.
Abbreviations: ND, Not done (insufficient RNA retrieved from posttreatment tumor biopsy); PR, partial remission; SD, stable disease; PD, progressive disease.

Toxicity

In general, toxicity of intratumoral injection of SCH 58500 was mild and mainly resulted from procedure-related discomfort. Nine patients experienced mild- to moderate-grade fever several hours after treatment, which spontaneously resolved no later than day 3. In addition, mild- to moderate-grade influenza-like symptoms, arthralgia, dyspnea, hypertension, and tachycardia were observed following SCH 58500 injection in one patient each. Adverse events that were considered to be related to the study treatment are summarized in Table 5. A transient increase in leukocyte counts was observed in eight patients without SCH 58500 dose dependency. There were five serious adverse events during the 28-day observation period: One patient treated at dose level 1 died on day 18 of the observation period. Postmortem examination revealed progressive disease and multiple pulmonary emboli as the cause of death. No signs of viral pneumonia were found, and no relation to study treatment could be established. Two patients experienced disease progression within the 28-day observation period. One patient experienced a hypertensive episode that required treatment. This patient had a past medical history of hypertension. One patient experienced diarrhea, malaise, and influenza-like symptoms, which required prolongation of his hospital stay. No adenovirus shedding was demonstrated in the sputum, urine, or stools of this patient, and the symptoms resolved on symptomatic treatment. There were no serious adverse vents resulting from bronchoscopies, percutaneous injections, or biopsies.

Virology studies

In all patients adenovirus shedding was assessed on a daily basis by ELISA, performed on sputum, urine, stools, and rectal swabs before treatment and for 72 hr after treatment or until negative. All patients were confirmed to be negtive for adenovirus excretion before treatment. Posttreatment viral shedding was observed in the sputum of one patient only on day 2 after treatment at dose level 3 (percutaneous). In another patient treated at dose level 1 falsely positive adenovirus shedding in urine and stool was found until day 6, owing to inappropriate use of the ELISA kit. In all other patients no evidence of adenovirus shedding could be demonstrated.

In all patients anti-SCH 58500 antibodies (i.e., anti-adenoviral antibodies) were detectable before treatment. A significant (≥twofold) increase in anti-SCH 58500 antibodies was observed in 11 of 15 patients following treatment with SCH 58500. Relative antibody levels increased by day 7 and remained at that level until day 28 (Fig. 2). There was no correlation between relative antibody levels observed and the dose of SCH 58500 administered. In one patient receiving a second dose of SCH 58500, development of anti-SCH 58500 antibodies following the first treatment did not prevent a second successful local wt *p53* gene transfer (Fig. 3).

DISCUSSION

The most common human cancers are strongly selected for mutations of the tumor suppressor gene *p53*, underscoring its central role in prevention of malignant transformation and tumor progression. Moreover, mutant *p53* status appears to reduce sensitivity toward commonly applied cytotoxic agents and radiotherapy (Levine, 1997). Thus, transfer of wild-type *p53* is an attractive target for cancer gene therapy. Numerous *in vitro* studies have demonstrated growth inhibition (Takahashi *et al.*, 1992), restoration of chemotherapy sensitivity (Fujiwara *et al.*, 1994b), as well as induction of apoptosis (Fujiwara *et al.*, 1993)

TABLE 4. DISEASE STATUS AND LONG-TERM FOLLOW-UP IN RELATION TO SCH 58500 DOSE LEVEL OF SIX PATIENTS WITH SUCCESSFUL WILD-TYPE *p53* GENE TRANSFER

			Day 28		
UPN	*Dose level (PFU)*	*Route*	*Treated*[a]	*Distant*[a]	*Follow-up*
005	10^8	B[b]	SD	PD	Died from PD
008	10^9	C[c]	PD	PD	Died from PD
009	10^9	C	SD	PD	Second treatment
010	10^9	B	SD[d]	PD	Died from PD
015	10^9	B	PD	PD	PD +2 months
012	10^{10}	C	SD	PD	SD +6 months

[a]Treated, treated tumor lesi n; distant, untreated tumor lesion(s).
[b]B, Bronch scopic intratumoral injection of 1 ml of SCH 58500 solution.
[c]C, CT-guided percutaneous intratumoral injection of 10 ml of SCH 58500 solution.
[d]Patient experienced local PR in treated tumor lesion at 2 months follow-up.
Abbreviation: UPN, Unique patient number.

TABLE 5. TREATMENT- AND/OR PROCEDURE-RELATED TOXICITIES IN RELATION TO SCH 58500 DOSE LEVEL[a]

	10^7 PFU (n = 3)[b]	10^8 PFU (n = 3)	10^9 PFU (n = 6)	10^{10} PFU (n = 3)
Hot flushes	II (1)	—	—	—
Flulike symptoms	—	—	II (1)	—
Arthralgia	—	—	—	I (1)
Fever	I (1)	I (2)	II (3)	II (3)
Dyspnea	—	—	II (1)	—
Hypertension	—	—	—	II (1)
Tachycardia	—	—	—	II (1)

[a]Roman numerals indicate maximal severity of adverse event (I, mild; II, moderate; III, severe; IV, life threatening). Digits in parentheses indicate number of patients experiencing the respective adverse event.
[b]*n*, Number of patients treated per dose level.

on wt *p53* gene transfer in lung cancer cells. Furthermore, in animal models of lung cancer a therapeutic effect of intratracheal instillation (Fujiwara *et al.*, 1994a) or intratumoral injection (Fujiwara *et al.*, 1994b; Wills *et al.*, 1994) of wt *p53*-containing expression vectors was noted. In these studies either retroviral (Cai *et al.*, 1993; Fujiwara *et al.*, 1993) or adenoviral (Zhang *et al.*, 1994; Harris *et al.*, 1996) vector constructs were applied with similar effects. An E1B-deficient adenoviral strain has been shown to replicate selectively in *p53* mutant tumor cells, leading to cytopathic effects at a low multiplicity of infection (Bischoff *et al.*, 1996; Heise *et al.*, 1997). This elegant approach used mutant *p53* as a target for virus-mediated tumor therapy without transferring wt *p53* itself.

An initial clinical pilot study reported induction of apoptosis and tumor regression in three of nine patients with advanced NSCLC treated by intratumoral injection of a fixed dose of a retroviral vector containing wt *p53* (Roth *et al.*, 1996). In that study vector DNA sequences were detected in some posttreatment tumor biopsies by DNA-PCR or by *in situ* hybridization. However, no transgene expression was demonstrated, limiting the interpretation of antitumoral and apoptotic effects observed in that trial.

In the present study intratumoral expression of vector-specific wt *p53* RNA as detected by RT-PCR was the primary end point. An adenoviral expression system was chosen because of its established safety in clinical trials, its organotropism, and its ability to transduce noncycling as well as dividing tumor cells (Zabner *et al.*, 1993; Crystal *et al.*, 1994, 1995; Hay *et al.*, 1995; Knowles *et al.*, 1995). In addition, the antitumoral efficacy of adenoviral gene transfer of wt *p53* has been demonstrated in several *in vitro* and preclinical *in vivo* models (Wills *et al.*, 1994; Harris *et al.*, 1996). Intratumoral transgene expression could be demonstrated in a manner suggestive of dose dependency in tumor samples taken within 48 hr posttreatment. Virus doses of 10^9 PFU were required to achieve detectable transgene expression in the majority of cases. The toxicity of the treatment in general was mild to moderate. Febrile reactions tended to increase with the vector dose administered. However, this inflammatory response did not prevent local wt *p53* gene transfer at higher virus doses. Moreover, evidence suggestive of short-term local tumor control was obtained. To minimize the risk for the patients only small posttreatment tumor samples could be obtained. Thus, the functional activity of the transgene in terms of induction of proteins encoded by genes downstream of *p53* could not be assessed. Antitumoral effects could therefore also be mediated by the adenovirus itself (Bischoff *et al.*, 1996) or by an anti-adenoviral immune reaction. In addition, an immune reaction against p53 peptides presented by tumor cells (Theobald *et al.*, 1995; Vierboom *et al.*, 1997) could have been augmented by intratumoral injection of SCH 58500.

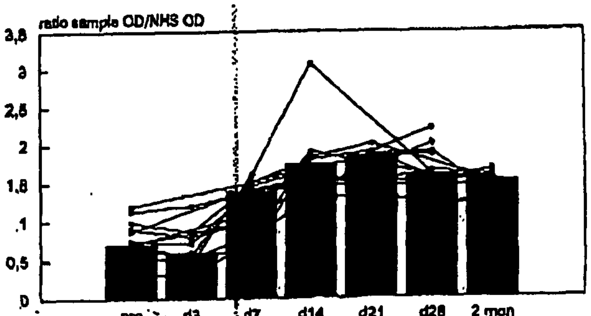

FIG. 2. Development of anti-SCH 58500 antibodies in relation to treatment. The boxes show the median values at each time point. OD, Optical density; NHS, normal human serum.

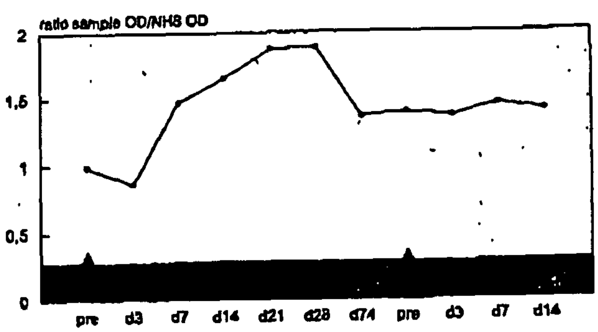

FIG. 3. Course of anti-SCH 58500 antibodies in a singl patient receiving two dosings of SCH 58500. The black triangles show the time points of treatment. The shaded area highlights the negative threshold of 0.28.

Clinical effects, such as local disease stabilization, as observed in this phase I study should be interpreted with caution. More importantly, intratumoral injection of SCH 58500 even at high viral doses was shown to be safe and feasible in patients with advanced NSCLC. In conclusi n, for the first time intratumoral transgene expression of wt *p53*, as obtained by adenovirus-mediated gene transfer, could be demonstrated in a clinical phase I study. An ongoing phase II study focuses on clinical efficacy of repetitive injections of SCH 58500 in conjunction with systemic cytotoxic chemotherapy.

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Exhibit 10

Adenovirus-mediated Wild-Type *p53* Gene Transfer as a Surgical Adjuvant in Advanced Head and Neck Cancers[1]

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ABSTRACT

A high incidence of locoregional failure contributes to the poor overall survival rate of around 50% for patients with squamous cell carcinoma of the head and neck (SCCHN). *In vitro* and *in vivo* preclinical work with adenovirus-mediated wild-type *p53* gene transfer using the recombinant *p53* adenovirus (Ad-*p53*) has shown its promise as a novel intervention strategy for SCCHN. These data have translated into Phase I and Phase II studies of Ad-*p53* gene transfer in patients with advanced, locoregionally recurrent SCCHN. The safety and overall patient tolerance of Ad-*p53* has been demonstrated. Of 15 resectable but historically noncurable patients in the surgical arm of a Phase I study, 4 patients (27%) remain free of disease, with a median follow-up time of 18.25 months. Surgical and gene transfer-related morbidities were minimal. These results provide preliminary support for the use of Ad-*p53* gene transfer as a surgical adjuvant in patients with advanced SCCHN. The implications of our findings for the management of SCCHN in general are discussed.

INTRODUCTION

The treatment of advanced primary human SCCHN[3] in the upper aerodigestive tract remains a major therapeutic challenge, despite advances in surgical and radiotherapeutic techniques. Locoregionally recurrent disease, which has a particularly dismal prognosis and few meaningful treatment options, remains the principal cause of death among patients with advanced SCCHN (1, 2). In addition, it has been shown that detection of clonal specific *p53* mutations at tumor margins in SCCHN is a predictor of local recurrence (3, 4). These molecular pathological advances suggest that despite adjuvant radiotherapy, residual disease (microscopic as well as histologically normal but genotypically abnormal) is a major problem in the treatment of patients with SCCHN. Our interest in developing new treatment strategies for SCCHN is generated by the humbling overall survival rate of approximately 50%, which has not changed over the last several decades (5).

Mutation of the *p53* tumor suppressor gene is one of the most common genetic alterations in human malignancy (6). Approximately 60% of human tumors are thought to possess mutation at the *p53* locus. Transient overexpression of the wild-type *p53* gene in various malignancies has been considered a potential molecular intervention strategy (7–12). This strategy is based on the role that wild-type *p53* plays as a tumor suppressor gene and an inducer of cell cycle arrest and apoptosis (6, 13–16). Our laboratory has focused on the potential of wild-type *p53* gene transfer as a strategy for the selective induction of apoptosis in SCCHN. The recombinant adenovirus Ad-*p53* has been used as the gene delivery tool in all of our preclinical studies (7–9). The tropism of the adenovirus for tissues of the upper aerodigestive tract, the ability to produce the adenovirus in high titers, and the efficiency of adenovirus-mediated gene transfer have made this vector an attractive tool for transient gene delivery.

In our preclinical studies with Ad-*p53*, transduction of wild-type *p53* into several different SCCHN cell lines induced apoptosis without adversely affecting normal cells (7, 8). We have also shown that Ad-*p53* reduces the growth of established tumors in xenograft models of SCCHN (8). Additionally, we have demonstrated that in a nude mouse xenograft model of microscopic residual disease, Ad-*p53* can prevent the establishment of tumors from subcutaneously deposited SCCHN cell lines in a dose-dependent fashion (7).

In our recently completed Phase I clinical trial of Ad-*p53* gene transfer in patients with advanced locoregionally recurrent SCCHN who were unsuccessfully treated with conventional therapy including radiotherapy, two treatment arms were established. Our previous report (17) demonstrated the feasibility and tolerance of Ad-*p53* administered to patients with nonresectable disease and to patients who could be surgically treated but were historically deemed incurable; tissue vector biodistribution was evaluated in this publication as well. In this current focused analysis with longer patient follow-up (median follow-up, 18.25 months), we report the potential antitumor activity and complications of Ad-*p53* in a surgical adjuvant setting (the surgical treatment arm), based on our Phase I experience.

MATERIALS AND METHODS

Study Subjects. Of the 33 total patients entered into the Phase I study, 15 patients with advanced locoregionally recur-

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[3] The abbreviations used are: SCCHN, squamous cell carcinoma of the head and neck; pfu, particle-forming unit; TdT, terminal deoxynucleotidyltransferase.

Table 1 Demographics of surgical treatment arm study participants

Patient no.	Age (yr)	Sex	*p53* genotype	Index tumor site	Prior treatments related to index tumor	Recurrent tumor site
1	31	F	Mutant	Tongue, floor of mouth	1. laser exc. L. tongue[a] 2. XRT to oral cavity 3. L. hemiglossectomy, mandibulectomy, MRND 4. 1 cycle CDDP & 5FU	Left face and neck
2	58	M	Mutant	Supraglottic larynx	1. supraglottic laryngectomy 2. completion laryngectomy, bilateral MRND 3. XRT to bilateral necks	Submental and submaxillary area
3	72	M	WT	Unknown primary	1. L. MRND 2. XRT to L. neck and supraclavicular region (5400 cGY)	Left neck
4	46	M	Mutant	Tongue base	1. L. RND and R. cervical node biopsy 2. XRT to L. and R. necks and tongue base (6600 cGy)	Tongue base
5	58	M	Mutant	Larynx	1. wide field laryngectomy 2. XRT to larynx (6300 cGy)	Hypopharynx
6	48	M	Mutant	Tongue base	1. XRT to tongue base, chemotherapy	Right superior larynx
7	76	F	Mutant	Supraglottic larynx	1. XRT to larynx, retropharyngeal and subdigastric nodes	Left supraclavicular area
8	53	M	WT	Unknown primary	1. R. RND 2. XRT to R. neck (5400 cGy) 3. XRT to submentum (4600 cGy) 4. 2 cycles cisplatin	Right submentum
9	56	M	Mutant	Larynx	1. verticle hemilaryngectomy 2. XRT to anterior neck and larynx (5500 cGy) 3. total laryngectomy	Neopharynx and peristomal region
10	49	F	NE	Left oral tongue	1. wide local exc. L. tongue 2. XRT to L. supraclavicular area (5040 cGy), L. neck (5600 cGy), and tongue and floor of mouth (3600 cGy)	Tongue
11	68	M	Mutant	Left tonsil	1. XRT to L. tonsil, L. upper neck, L. supraclavicular fossa (7000 cGy)	Left tonsil
12	37	M	Mutant	Left oral tongue	1. hemiglossectomy 2. re-excision L. tongue 3. XRT to tongue (6400 cGy) 4. L. MND	Left tongue base
13	34	F	NE	Floor of mouth and submentum	1. wide local exc. tongue and floor of mouth, L. MRND 2. 2 cycles of Taxol, ifosphamide, cisplatin 3. XRT and 2 cycles of 5FU and cisplatin	Left tongue and floor of mouth
14	56	M	Mutant	Right hypopharynx	1. 2 cycles of 5FU and cisplatin 2. partial laryngopharyngectomy, R. RND 3. XRT to R. neck (6300 cGy)	Right hypopharynx
15	73	F	Mutant	Left buccal mucosa	1. exc. L. buccal lesion 2. exc. L. retromolar trigone 3. L. hemimandibulectomy, L. MRND 4. XRT to L. cheek, face, and neck (4500 cGy) 5. XRT boost (900 cGy) to L. cheek	Left buccal mucosa

[a] L., left; R., right; exc., excision; XRT, radiation therapy; CDDP, cis-diamminedichloroplatinum; 5FU, 5-fluorouracil; MRND, modified radical neck dissection; RND, radical neck dissection; WT, wild-type; NE, could not be evaluated. Recurrent tumor site refers to the recurrent lesion that was treated in this Phase I trial.

rent or refractory SCCHN were placed into the surgical treatment arm. These 15 patients are the subjects of this report. For this report, we also examined biopsy samples of tumor margin and untreated adjacent normal tissues from a representative nonsurgical patient for evidence of apoptosis as well as expression of the wild-type p53 and $p21^{Waf1}$ gene products.

Patients typically had multiple treatments for either refractory or locoregionally recurrent disease before study entry (Ta-

ble 1). All patients had previously received radiotherapy at some point during their treatment. Entry into the surgical treatment arm required only that the tumor could be resected to microscopic residual disease (without resection of the internal carotid artery), but resection offered little or no opportunity for cure as determined by the Multidisciplinary Head and Neck Oncology Treatment Planning Committee at The University of Texas M. D. Anderson Cancer Center. There were 10 males and 5 females, with a mean patient age of 54.3 years. Tumor *p53* genotype was analyzed (by direct sequencing) for each patient, although a mutant genotype was not a prerequisite for study entry. Patients were required to practice contraception while in the study, and women of child-bearing age had to have negative pregnancy tests. A detailed description of the 15 subjects can be found in Table 1. The study was reviewed and approved by the Institutional Surveillance Committee of The University of Texas M. D. Anderson Cancer Center, the NIH Recombinant DNA Advisory Committee, and the Food and Drug Administration. Informed consent was obtained from all patients before study entry, with emphasis placed upon the investigational nature of the study and the absence of therapeutic intent.

Recombinant Adenovirus. The recombinant adenovirus Ad-*p53* was used to directly introduce the wild-type *p53* gene into all subjects. Preparation of the recombinant adenovirus was described previously (18). Ad-*p53*, also designated as INGN201, is a replication-defective adenovirus serotype 5 vector with a cytomegalovirus-promoted *p53* cDNA insert replacing the E1 region of the vector. Ad-*p53* is a BL-2 agent and was handled with the appropriate level of biological containment. Ad-*p53* was produced by Magenta, Inc. (now MA Biosciences, Rockville, MD) and Introgen Therapeutics (Houston, TX) and stored at −80°C at concentrations of 2×10^{10} to 3×10^{10} pfu/ml in PBS supplemented with 10% glycerol. Ad-*p53* was thawed and diluted in PBS at 4°C within 2 h of use.

Administration of Ad-*p53*. All Ad-*p53* was administered on an inpatient basis under strict aseptic conditions. Ad-*p53* was delivered to sites of disease recurrence only. There were three Ad-*p53* intervention approaches/patient: (*a*) preoperative; (*b*) intraoperative; and (*c*) postoperative.

Ad-*p53* was given in escalating doses to determine a maximum tolerated dose for this treatment strategy. The Ad-*p53* dose did not vary throughout each patient's treatment (Table 2). Doses started at 1×10^6 pfu and were increased in log increments until 1×10^9 pfu was reached and then increased in one-half log increments until 1×10^{11} pfu was reached.

The preoperative Ad-*p53* administration consisted of direct tumor injections given three times weekly for 2 consecutive weeks (six treatments overall). The preoperative injection volumes were based on the estimated volume of the injected mass and the number of injection sites. Ad-*p53* was administered using 27-gauge needles and 3–10-ml syringes, depending on the volume injected. Ad-*p53* was injected directly into tumors by inserting the needle to the tumor depth and injecting upon withdrawal. Ad-*p53* was diluted in a volume of PBS concordant with the number of tumor injections to be performed. Generally, we injected about 0.5 ml of vector solution at 1-cm (surface area) tumor increments. Thus, a very large tumor required the appropriate amount of vector to be diluted in a larger volume of PBS. Tumor maps were generated depicting the injection sites so that these areas could be reinjected. A typical tumor map for a recurrent oral tongue lesion is shown in Fig. 1.

Seventy-two h after the last Ad-*p53* intratumoral injection, patients had their surgery. At the time of surgery, after total gross tumor removal and just before closure, another dose of Ad-*p53* (diluted to 10 ml in PBS) was delivered by injection to the surgical sites where microscopic residual disease was presumed to be present, including mucosal margins of the resected neoplasms (Fig. 2). A small amount of this dose was saved and administered liberally (a vector wash) to the tumor bed via a syringe and left in contact for 60 min before wound closure.

Seventy-two h after surgery, the patients received the final Ad-*p53* administration (again diluted to 10 ml in PBS) via retrograde instillation through wound catheters that had been placed intraoperatively in the areas of presumed microscopic residual disease. Clamps were used to prevent efflux of the Ad-*p53* for 1 h. The drains were removed 24–48 h after the postoperative instillation.

Statistical Analysis of Patient Outcome. Kaplan-Meier disease-specific survival and disease-free intervals were analyzed for all 15 patients entered into the surgical arm of the study. The time of study entry was the day of the first preoperative Ad-*p53* administration. All patients were macroscopically free of disease after surgical resection.

Patient Monitoring. Because the treatment of patients with Ad-*p53* was within the context of a Phase I clinical trial, diligent patient monitoring for the detection of untoward and toxic effects was obligatory. Surgical complications as well as potential Ad-*p53*-related toxic effects were recorded. Vital signs were recorded, performance status was evaluated, and chest X-rays and hematology and blood chemistry testing were performed daily. Patients were closely monitored for 2 h after each Ad-*p53* administration.

Detection of Wild-Type p53 and p21^{Waf1} Gene Product Expression and Apoptosis. Biopsy samples taken from the tumor margins of a representative nonsurgical patient were analyzed 48 h after Ad-*p53* delivery (10^6 pfu) to the tumor. This immunohistochemical analysis examined the expression of the wild-type p53 gene product and the gene product of the downstream p53-transactivated gene *p21^{Waf1}* (19) via an avidin-biotin-peroxidase complex method (20). The DO-1 anti-p53 mouse monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and the anti-p21^{Waf1} mouse monoclonal antibody (Oncogene, Uniondale, NY) were used for all p53 and p21^{Waf1} immunohistochemical studies, respectively. Standard H&E staining as well as TdT end-labeling to detect apoptotic cells were performed on similarly prepared tumor margin biopsy samples 48 h after Ad-*p53* delivery to the tumor. TdT end-labeling was performed with the ApoTag Plus kit (Oncor, Gaithersburg, MD) according to the manufacturer's instructions. All of the these studies were matched with biopsy samples taken from adjacent uninjected grossly normal tissues of the same patient 48 h after Ad-*p53* delivery to the tumor.

RESULTS

Patient Outcome. The Kaplan-Meier disease-specific survival curve for the patients enrolled in the surgical arm of the

Table 2 *Ad-p53* and surgical treatment and related complications of surgical treatment arm study participants

Patient no.	Ad-*p53* dose per treatment 1×10^x pfu	Adenovirus complications	Surgical treatment of recurrence	Surgical complications	Current disease status
1	6	Headache with first preoperative injection	Left maxillectomy and mandibulectomy, bilateral NDs,[a] total laryngectomy, partial pharyngectomy, latisimus free flap and bilateral pectoralis flap reconstruction	Postoperative fever with positive blood cultures for *Staphylococcus*	DOD
2	6	Erythema at preoperative injection site	Total glossectomy, total pharyngectomy, bilateral NDs, resection anterior neck skin, right pectoralis flap reconstruction	Intraoperative bradycardia and atrial flutter, electrolyte imbalance	DOD
3	7	None	Left extended RND, left pectoralis flap reconstruction	None	NED
4	7	Pain with preoperative injections	Total laryngectomy, total glossectomy, right MRND, marginal mandibulectomy, left verticle rectus myocutaneous flap reconstruction	None	DOD
5	8	None	Total pharyngectomy, cervical esophagectomy, completion thyroidectomy, bilateral MRND, mediastinal nodal dissection, free jejunum reconstruction	Anemia, electrolyte imbalance, confusion, fever, mild respiratory insufficiency, acute renal insufficiency	NED
6	8	None	Total laryngectomy, right MRND, left RND, partial pharyngectomy, subtotal thyroidectomy, total glossectomy, free rectus flap and pectoralis flap reconstruction	Anemia, electrolyte imbalance, acute renal insufficiency	DOD
7	9	Tenderness at preoperative injection site	Left extended RND, pectoralis flap reconstruction	Anemia, electrolyte imbalance, aspiration pneumonia	DOC
8	9.5	Fever during early preoperative injections, pain with preoperative injections	Right ND	None	DOD
9	10.5	Sore throat, increased dysphagia, sinus congestion, and headache during preoperative injections	Total pharyngectomy, total thyroidectomy, bilateral MRND, free jejunum reconstruction	Anemia, electrolyte imbalance, ascites, hypothyroidism, pneumatosis intestinalis	DOD
10	10.5	Fever, sore throat, headache, and increased odynaphagia and dysphagia during preoperative injections	Partial glossectomy, hemimandibulectomy, left MRND, free fibula osseo-cutaneous flap reconstruction	Anemia, electrolyte imbalance, hypertension, pneumonia, delayed cervical wound healing	DOD
11	10.5	Fever during preoperative injections, and pain associated with injections	Left partial pharyngectomy, left partial mandibulectomy, left RND, right verticle rectus free flap reconstruction	Anemia, electrolyte imbalance, pleural effusion, pneumonia and respiratory failure, hypertension, cellulitis left neck	DOC
12	11	Pain after preoperative injections, throat swelling after first preoperative injection	Total glossectomy, total laryngectomy, partial pharyngectomy, partial mandibulectomy, bilateral ND, free transverse rectus abdominis flap reconstruction	Anemia, electrolyte imbalance and hypovolemia, fever	DOD

Phase I Ad-*p53* clinical trial is shown in Fig. 3. Median survival was 12.4 months. Currently, four patients are alive with no evidence of disease (at 29.1, 23.8, 11.5, and 12.7 months). One patient is alive with disease (at 13.1 months), and eight patients have died of disease. Two patients died of unrelated causes (at 13.4 and 4.8 months). The current disease status of each study participant is shown in Table 2.

The median disease-free interval was 3.9 months for the nine patients enrolled in the surgical treatment arm whose disease recurred. The four patients without evidence of disease (disease free at 29.1, 23.8, 11.5, and 12.7 months) and the two patients who were without evidence of disease at the time of death (at 13.4 and 4.8 months) were not included in this calculation.

Table 2 Continued

Patient no.	Ad-*p53* dose per treatment 1×10^x pfu	Adenovirus complications	Surgical treatment of recurrence	Surgical complications	Current disease status
13	11	Fever after first two preoperative injections, erythema and induration at preoperative injection sites, headaches postinjection	Total glossectomy, total laryngectomy, partial mandibulectomy, bilateral ND, free rectus flap reconstruction	Anemia, electrolyte imbalance, breakdown of flap reconstruction	NED
14	11	Fever and headache following preoperative injections	Total laryngopharyngectomy, left MRND, free jejunum reconstruction	Electrolyte imbalance, respiratory failure, hypothyroidism and hypoparathyroidism	NED
15	11	Fever after first preoperative injection, erythema and induration at preoperative injection site	Resection left buccal mucosa, partial maxillectomy, partial mandibulectomy, infratemporal fossa resection, left MRND, free flap reconstruction	Electrolyte imbalance, flap hematoma, agitation and confusion	AWD

[a] ND, neck dissection; AWD, alive with disease; DOC, died of other causes; DOD, died of disease; NED, no evidence of disease; RND, radical neck dissection; MRND, modified radical neck dissection.

Surgical Complications. Despite the extensive prior treatments and often tremendous tumor burdens, surgical complications in the context of Ad-*p53* administration were relatively minor among the 15 study subjects, considering the extent of resection in most cases. There was one instance of delayed wound healing (patient 10) and one instance of flap breakdown (patient 13) requiring operative revision. There were no fistulas or wound infections. A detailed list of the surgical procedures for each patient, along with related complications, is provided in Table 2. The most common complications were electrolyte imbalance (usually consisting of transient hypokalemia or hypomagnesemia due to prolonged anesthesia; 11 of 15 patients), anemia (8 of 15 patients), pneumonia (3 of 15 patients), acute renal insufficiency (2 of 15 patients), and transient hypothyroidism (2 of 15 patients). All complications resolved with appropriate fluid or pharmacological intervention or both.

Ad-*p53*-related Complications. The dose of Ad-*p53* that was administered to each patient is indicated in Table 2, along with a detailed list of treatment-related sequelae. All Ad-p*53*-related complications occurred during the preoperative administrations and were mild. All patients were able to tolerate the full course of Ad-*p53* interventions (preoperative, intraoperative, and postoperative). As can be seen in Table 2, Ad-*p53*-related complications were more frequent at the higher viral doses ($\geq 1 \times 10^9$ pfu). Fever after Ad-*p53* administration was the most common finding, occurring in six patients. Fever was not observed in patients who received less than 1×10^9 pfu/dose and was only transiently observed after the first injection or the first and second injections during preoperative administration. Fevers ranged from 38.1°C in patient 11 to 39.4°C in patient 10. Pain at the site of injection was also a frequent finding, occurring in five patients. This sequela was believed to be related to the cold temperature of the injected Ad-*p53* solution. In patients 9, 10, 12, and 13, mild, transient, flu-like symptoms were observed early in their preoperative Ad-*p53* administration courses.

Gene Product Expression and Induction of Apoptosis. Dark green positive immunohistochemical stainings for the wild-type p53 gene product (Fig. 4*D*) and the $p21^{Waf1}$ gene product (Fig. 4*F*) were demonstrated at the tumor margins of an Ad-*p53*-treated tumor from a representative nonsurgical patient. Matched samples from adjacent untreated normal tissue (Fig. 4, *C* and *E*) stained negatively. Only mild suprabasal detection of endogenous p53 was detected in the untreated tissue (Fig. 4*C*). It should be noted that the wild-type p53 gene product was detected despite the presence of a rigorous immune infiltrate (and systemic anti-adenovirus antibody titer; data not shown) seen on a posttreatment H&E-stained section from the tumor margin (Fig. 4*B*). Fig. 3*H* shows the brown-stained apoptotic tumor cells in the submucosa (by TdT end-labeling assay) present in a biopsy sample of the tumor margin after Ad-*p53* injection, relative to the matched biopsy of adjacent untreated normal tissue (Fig. 4*G*).

DISCUSSION

Because of its propensity for locoregional recurrence and poor survival, SCCHN remains a devastating disease, despite treatment advances (1, 2, 5). A major factor leading to locoregional recurrence of SCCHN is microscopic residual disease after definitive surgery, radiotherapy, chemotherapy, or any combination of the three. Even histologically "normal" tissue at the margins of tumor resection can harbor molecular characteristics that portend disease recurrence (3, 4).

The situation of patients with locoregionally advanced SCCHN who have unsuccessfully undergone other therapies, including radiotherapy, is particularly problematic. Additional chemotherapy does not seem to offer a significant survival advantage to these patients (21), and they have few viable treatment options, even when tumors are surgically resectable. Thus, we selected this population of patients for our Phase I clinical trial of Ad-*p53* gene therapy. The known role of *p53* as a tumor suppressor gene and an inducer of cell cycle arrest and apoptosis in mammalian cells (6, 13–16), as well as our encouraging preclinical *in vitro* and *in vivo* animal findings with Ad-*p53* in SCCHN (7–9), made this an attractive treatment strategy.

As indicated earlier, the Phase I study of patients with

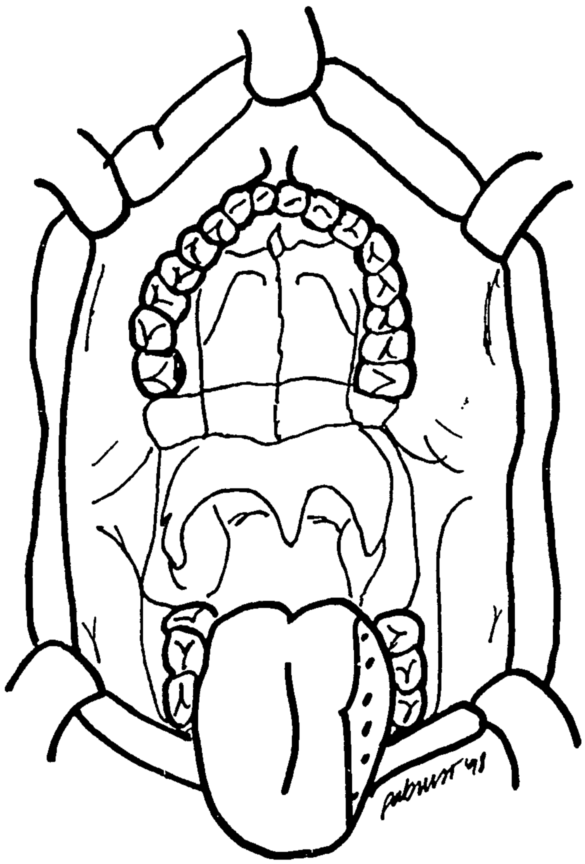

Fig. 1 Example of a typical tumor map for a left tongue carcinoma. Note the incremental markings along left tongue lesion indicating sites where Ad-*p53* is injected.

Fig. 2 Intraoperative delivery of Ad-*p53* to the tumor bed. Ad-*p53* is being injected into the tumor margins before a vector wash of the tumor bed.

advanced locoregionally recurrent SCCHN revealed that Ad-*p53* gene transfer is safe and well tolerated (17). Furthermore, in the current analysis, apoptosis and expression of the wild-type p53 and p21$^{\mathrm{Waf1}}$ (a downstream p53-transactivated gene) gene products were demonstrated in tumor margin biopsy samples taken from a representative nonsurgical patient after Ad-*p53* delivery. The findings with regard to median survival in the surgical arm of the study (Ad-*p53* delivered as an adjuvant to surgical therapy) prompted the current report, although our sample size was small, and thus the results should not be overinterpreted. The median survival for these patients (12.4 months) was about 60% longer than that found in chemotherapy trials for similar patients (21). Furthermore, the median disease-free interval of 3.9 months among those patients whose disease recurred suggests that this trial was not preselecting a favorable patient population. The observations made with regard to potential antitumor activity among patients with resectable tumors is encouraging as we proceed with the international Phase II evaluation of Ad-*p53* gene transfer in patients with SCCHN. Recurrence rates and mortality are higher in patients with molecular evidence of residual disease (as determined by PCR-based assay of *p53* mutation) at tumor margins (1, 2). Thus, the use of Ad-*p53* as an adjuvant modality in surgical wound beds may lower those rates.

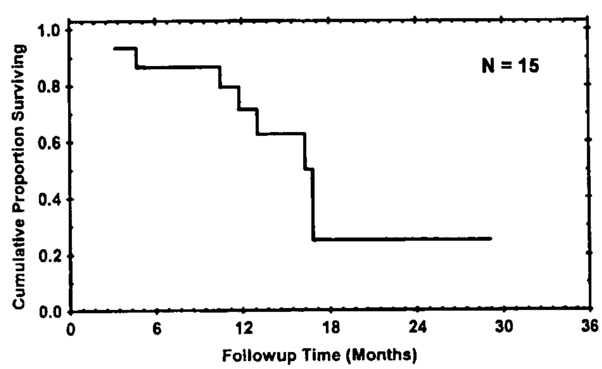

Fig. 3 Kaplan-Meier survival curve for patients in the surgical treatment arm.

There are several implications of our findings. Given the low toxicity of Ad-*p53*, this agent may be applied as an adjuvant therapy after primary definitive treatment of advanced lesions (or early lesions), as indicated above. Furthermore, Ad-*p53* gene transfer may be efficacious in dysplastic lesions because *p53* mutations have been found in head and neck premalignancies

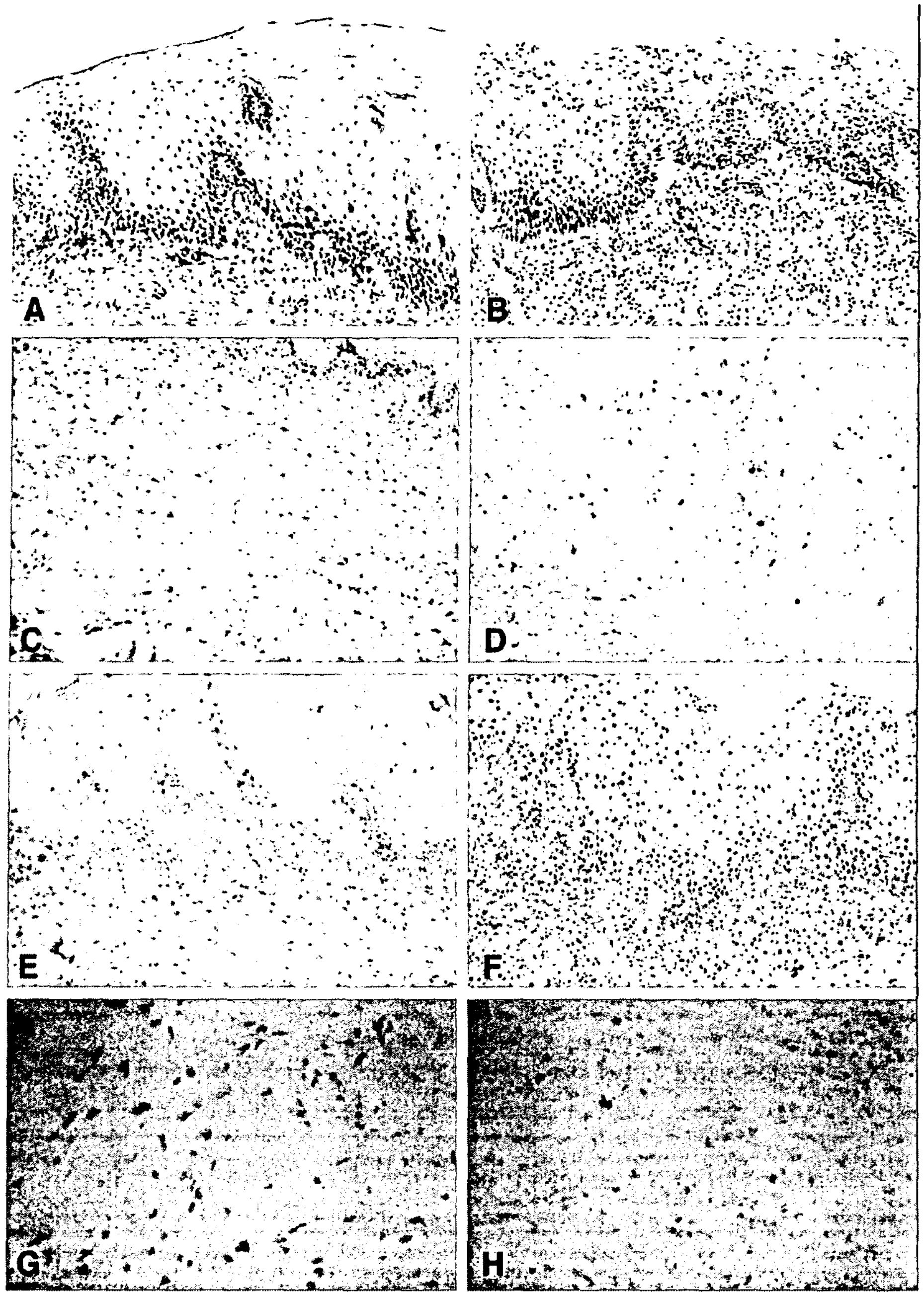

Fig. 4 Immunohistochemical, H&E, and TdT end-labeling analyses of biopsies taken from the tumor margins of a representative nonsurgical patient 48 h after Ad-*p53* delivery to the tumor. In tumor margin biopsy samples, immunohistochemical staining for expression of the wild-type p53 gene product (*D*) and p21$^{\mathrm{Waf1}}$ gene product (*F*) is shown 48 h after Ad-*p53* delivery to the tumor. *C* (immunostained for expression of the wild-type p53 gene product) and *E* (immunostained for expression of the p21$^{\mathrm{Waf1}}$ gene product) show matched biopsy samples taken from untreated normal tissues 48 h after Ad-*p53* delivery to the tumor. In tumor margin biopsy samples, H&E staining (*B*) and TdT end-labeling (*H*) are shown 48 h after Ad-*p53* delivery to the tumor. *A* (stained with H&E) and *G* (end-labeled with TdT) show matched biopsy samples taken from untreated normal tissues 48 h after Ad-*p53* delivery to the tumor.

(22). Finally, Ad-*p53* gene therapy may be applied in combination with radiotherapy or chemotherapy because enhanced antitumor activity has been seen in such combination treatment models in preclinical studies (23, 24).

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Exhibit 9

ARTICLE

Adenovirus-Mediated p53 Gene Transfer in Advanced Non-Small-Cell Lung Cancer

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Background: **Preclinical studies in animal models have demonstrated tumor regression following intratumoral administration of an adenovirus vector containing wild-type p53 complementary DNA (Ad-p53). Therefore, in a phase I clinical trial, we administered Ad-p53 to 28 patients with non-small-cell lung cancer (NSCLC) whose cancers had progressed on conventional treatments.** *Methods:* **Patients received up to six, monthly intratumoral injections of Ad-p53 by use of computed tomography-guided percutaneous fine-needle injection (23 patients) or bronchoscopy (five patients). The doses ranged from 10^6 plaque-forming units (PFU) to 10^{11} PFU.** *Results:* **Polymerase chain reaction (PCR) analysis showed the presence of adenovirus vector DNA in 18 (86%) of 21 patients with evaluable posttreatment biopsy specimens; vector-specific p53 messenger RNA was detected by means of reverse transcription–PCR analysis in 12 (46%) of 26 patients. Apoptosis (programmed cell death) was demonstrated by increased terminal deoxynucleotide transferase-mediated biotin uridine triphosphate nick-end labeling (TUNEL) staining in posttreatment biopsy specimens from 11 patients. Vector-related toxicity was minimal (National Cancer Institute's Common Toxicity Criteria: grade 3 = one patient; grade 4 = no patients) in 84 courses of treatment, despite repeated injections (up to six) in 23 patients. Therapeutic activity in 25 evaluable patients included partial responses in two patients (8%) and disease stabilization (range, 2–14 months) in 16 patients (64%); the remaining seven patients (28%) exhibited disease progression.** *Conclusions:* **Repeated intratumoral injections of Ad-p53 appear to be well tolerated, result in transgene expression of wild-type p53, and seem to mediate antitumor activity in a subset of patients with advanced NSCLC. [J Natl Cancer Inst 1999;91:763–71]**

The p53 gene (also known as TP53) encodes a 593-amino acid phosphoprotein that plays a critical role in cell cycle regulation and control of apoptosis *(1–3)*. p53 gene mutations have been associated with tumor progression and the development of chemotherapy and radiation therapy resistance *(4–6)*. The development of gene transfer technology has allowed the transduction of cancer cells with wild-type p53 (wt-p53). Intratumoral injection of retroviral or adenoviral wt-p53 constructs in animal models results in tumor regression in a variety of different tumor histologies, including non-small-cell lung cancer (NSCLC), leukemia, glioblastoma, and breast, liver, ovarian, colon, and kidney cancers *(7–13)*. Furthermore, Roth et al. *(14)* demonstrated the safety and feasibility of using a retroviral wt-p53 construct in patients with advanced NSCLC. In that trial, tumors regressed in three of seven evaluable patients after bronchoscopic or computed tomography (CT)-guided injection of retroviral wt-p53.

The clinical use of retrovirus vectors is limited, however, by difficulties in transducing nonreplicating cells and producing high titers of virus. Adenoviruses, on the other hand, are large, double-stranded DNA viruses with a tropism for lung cancer cells *(15)*. Furthermore, they are capable of transducing nonreplicating cells and can be grown to high titers *in vitro*, which allows for their potential clinical utility *(16)*. We, therefore, designed a phase I clinical trial using an adenovirus vector containing wt-p53 complementary DNA (cDNA) to treat patients with advanced NSCLC whose cancers had progressed on conventional treatments.

Patients and Methods

Protocol approval. The protocol used was approved by the Biosafety and Surveillance Committees/Institutional Review Board of the participating institutions, the Recombinant DNA Advisory Committee of the National Institutes of Health, and the U.S. Food and Drug Administration *(17)*.

Gene transfer vector. The construction and generation of Ad-p53 were reported previously *(18)*. Briefly, E1-deleted replication-defective recombinant

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See "Notes" following "References."

adenovirus was constructed with the use of a modified type 5 adenovirus genome. The cytomegalovirus (CMV) promoter was used to drive transcription of human wt-p53 cDNA. Ad-p53 was supplied by Introgen Therapeutics, Inc.

Eligibility criteria and treatment protocol. Patients with histologically proven NSCLC were enrolled in the trial. All patients had unresectable tumors and either were unable to receive primary external beam radiation therapy or had a recurrence after such therapy. Patients were also eligible if they did not respond to or relapsed after chemotherapy. Patients had either an endobronchial tumor that was accessible by the bronchoscope with some clinical evidence of bronchial obstruction, advanced local–regional cancer that was unresectable, or isolated metastases whose regression or stabilization would offer potential benefit to the patient. Written informed consent was obtained from all patients stating that they were aware of the investigational nature of this study, in keeping with institutional policies. Pretreatment tumor biopsies demonstrating overexpression of the p53 protein by the criteria of Nishio et al. *(19)* were required for entry in the protocol. Mutations in the p53 gene were identified by single-strand conformation polymorphism (SSCP) analysis and DNA sequencing of a tumor biopsy specimen as described previously *(20)*. All mutations were reconfirmed by repeated sequencing or SSCP analysis of a second independent polymerase chain reaction (PCR) reaction. Patients were not treated on protocol until 4 weeks after completing systemic or local therapy. The preclinical safety studies and treatment protocol have been described previously *(21,22)*. Ad-p53 was diluted in phosphate-buffered saline and administered by needle injection directly into the tumor, either percutaneously or bronchoscopically. For lesions at least 4 cm in the largest diameter, the final volume given was 10 mL; for lesions with a diameter of less than 4 cm, the final volume given was 3 mL. The entire volume was injected at a single site. Patients were treated monthly for up to six injections of Ad-p53. Doses were escalated from 10^6 plaque-forming units (PFU) to 10^{11} PFU in one-half or one log increments (Table 1).

Toxicity and response. The toxic effects of therapy were evaluated according to the National Cancer Institute's Common Toxicity Criteria *(23)*. Response to therapy was assessed by chest roentgenogram or CT scans before each course of treatment, by use of standard criteria *(14)*. Responses were confirmed by two evaluations taken 4 weeks apart. Patients were evaluable for response if they had received at least one course of therapy followed by an appropriate radiograph to document response. Response criteria were defined as follows: 1) complete response, i.e., disappearance of all clinical evidence of tumor by physical examination, roentgenography, and CT (or magnetic resonance imaging) scans for a minimum of 4 weeks; 2) partial response, i.e., a 50% or greater decrease in the sum of the products of the perpendicular diameters of measurable lesions for a minimum of 4 weeks and no simultaneous increase of at least 25% in the size of any lesion or the appearance of any new lesion; 3) stable disease, i.e., any variation of the indicator lesion not meeting the criteria of a complete or partial response or progression; and 4) progressive disease, i.e., an increase of at least

Table 1. Characteristics of patients with non-small-cell lung cancer (NSCLC), characteristics of their tumors, prior therapy, and response to treatment with intratumoral injection of Ad-p53, an adenovirus vector carrying wild-type p53 complementary DNA

Patient*	Age, y	Sex	Histology†	Prior surgery	Prior chemotherapy courses‡	Prior radiation therapy§	Injection site‖	Baseline measurement, cm	Method of injection¶	Viral dose, plaque-forming units	No. of courses	Response#
A	45	Female	Squamous	No	5	6300	Lung	4 × 6	CT	10^6	4	Stable
B	61	Male	Squamous	Yes	2	12 420	Lung	10 × 9	CT	10^6	2	Stable
C	75	Female	Adeno	No	4	None	Bronchus	5 × 6	Bronch	10^6	1	Progression
D	58	Male	Squamous	No	9	6000	Subcarinal nodes	4 × 2	CT	10^7	3	Progression
E	43	Female	Adeno	Yes	3	7500	Liver	2 × 2	CT	10^7	2	Progression
F	58	Female	Large cell	Yes	0	6000	Axilla	3 × 6	CT	10^7	1	Not evaluable**
G	42	Male	Adeno	No	0	None	Liver	4.5 × 6	CT	10^8	3	Stable
H	61	Male	Adeno	No	3	6120	Liver	5 × 6	CT	10^8	4	Stable
I	65	Female	Sarco	No	3	None	Chest wall	6.5 × 5	CT	10^8	2	Stable
J	71	Female	Squamous	Yes	0	6480	Lung	5 × 5	CT	10^9	2	Progression
K	72	Female	Large cell	No	6	6600	Lung	4 × 5	CT	10^9	6	Partial response
L	78	Male	Adeno	Yes	2	6000	Liver	4 × 5	CT	10^9	3	Stable
M	68	Female	Squamous	No	2	Yes	Lung	3.5 × 5	CT	3×10^9	4	Stable
N	72	Female	Adeno	Yes	0	8000	Lung	6.5 × 7	CT	3×10^9	2	Stable
O	70	Female	Adeno	Yes	2	6000	Bronchus	ND††	Bronch	3×10^9	6	Partial response
P	52	Male	Adeno	No	6	6000	Bronchus	3.9 × 6.3	Bronch	3×10^9	2	Stable
Q	65	Female	Adeno	Yes	0	11 000	Lung	3.5 × 3.5	CT	10^{10}	5	Stable
R	68	Female	Large cell	No	0	None	Lung	5 × 5	CT	10^{10}	2	Stable
S	55	Male	Squamous	No	3	6000	Lung	5 × 5	CT	10^{10}	5	Stable
T	46	Female	Adeno	Yes	4	4000	Lung	5.3 × 5.3	CT	3×10^{10}	2	Progression
U	71	Male	Squamous	Yes	2	13 914	Bronchus	ND††	Bronch	3×10^{10}	1	Not evaluable**
V	67	Female	Adeno	No	3	None	Lung	4 × 2.5	CT	3×10^{10}	6	Stable
W	55	Female	Adeno	No	4	None	Lung	3.8 × 4.5	CT	10^{11}	3	Progression
X	75	Male	Squamous	No	5	6300	Lung	6.5 × 3	CT	10^{11}	6	Stable
Y	61	Male	Squamous	No	2	6800	Bronchus	ND††	Bronch	10^{11}	1	Not evaluable**
Z	74	Male	Large cell	No	0	8000	Liver	9.3 × 8.3	CT	10^{11}	2	Stable
AA	62	Female	Squamous	No	2	6300	Lung	5 × 6.5	CT	10^{11}	3	Stable
BB	67	Female	Adeno	No	5	None	Chest wall	2 × 4	CT	10^{11}	1	Progression

*Sequential letters were assigned during manuscript editing to ensure confidentiality of patients; the letters do not represent identifiers used during the trial.

†All tumors were histologically confirmed before treatment as viable NSCLC; squamous = squamous cell carcinoma; adeno = adenocarcinoma; large cell = large-cell carcinoma; and sarco = sarcomatoid subset of NSCLC.

‡Number of chemotherapy courses given at least 3 months prior to Ad-p53 treatment.

§Centigray (cGy) of external-beam radiation therapy given at least 3 months prior to Ad-p53 therapy.

‖Location of indicator lesion injected with Ad-p53.

¶Mode of delivery of intratumoral Ad-p53 during monthly treatments; CT = computed tomography (CT)-guided injection; Bronch = bronchoscopic injection.

#Complete response = complete disappearance of tumor as judged by CT scan and physical examination for a minimum of 4 weeks; partial response = decrease ⩾50% in size of tumor for minimum of 4 weeks; progression = increase ⩾25% in size of tumor; stable = any variation in size not meeting criteria of complete response, partial response, or progression.

**Not evaluable because patients died of non-treatment-related causes prior to 30-day follow-up CT scan.

††ND = not determined because it was an endobronchial tumor.

25% in the size of a bidimensionally or unidimensionally measurable lesion, a clinically significant increase in the size of noninjected disease, or the appearance of any unequivocal new lesion.

Terminal deoxynucleotide transferase (TdT)-mediated biotin uridine triphosphate nick-end labeling (TUNEL) assay for DNA fragmentation. Pretreatment (immediately before) and posttreatment (3 days after) tumor biopsy specimens were obtained by core biopsies of the vector-injected tumor after each course of treatment (Fig. 1). The TUNEL assay was a modification of a previously described technique *(24,25)*. Slides were counterstained with 0.4% methylene green. Negative controls were performed by omitting TdT from the buffer solution, and positive controls included analysis of deoxyribonuclease-treated slides. Corresponding hematoxylin–eosin slides were evaluated for the presence of an inflammatory cell infiltrate and were graded on a scale of 1–4. All histology slides were coded and read blinded by a single observer, who had no knowledge of the patients, biopsy sequence, or the clinical status.

Reverse transcription (RT)–PCR and DNA PCR. Total RNA extraction, RT, PCR amplification, and blot hybridization were performed by a modification of a previously described technique *(26,27)*. A nested PCR procedure was used, with vector-specific primers CMV3 (5′-GGTGCATTGGAACGCGGATT-3′) and Rev Ex3 (5′-CAAATCATCC ATTGCTTGGGA-3′) used for the first round and CMV3 and RN3 (5′-GGGGACAGAACGTTGTTTTC-3′) used for the second round of amplification. Human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (GAPDH-S [5′-CAGCCGAGCCACATC-3′] and GAPDH-AS [5′-TGAGGCTGTTGTCATACTTCT-3′]) were used as a positive reaction control, and saline was used as a negative control. All samples underwent PCR amplification without prior reverse transcriptase treatment to test for completeness of deoxyribonuclease digestion. RT–PCR amplification was not performed if DNA PCR was negative for adenoviral DNA or biopsy samples were inadequate for RNA evaluation. DNA PCR was performed on DNA extracted from biopsy specimens by use of the primers described above.

Statistical methods. Because of the small sample size, descriptive statistics were reported in tabular form. Ninety-five percent confidence intervals (CIs) were constructed to estimate the pretreatment apoptotic index (AI). Patients were considered to have increased apoptotic activity if the posttreatment AI was greater than the upper end of the 95% CI of the pretreatment AI. The overall survival was calculated by the Kaplan–Meier estimate. Survival time was defined as time from study entry to death or date of last follow-up.

RESULTS

Patient and Tumor Characteristics

Twenty-eight NSCLC patients (17 females and 11 males) with a median age of 65 years (range, 42–78 years) were entered in the study (Table 1) beginning October 24, 1995, until December 8, 1997. Partial information on 12 of these patients was reported previously *(28)*, together with information on nine patients who received Ad-p53 and cisplatin in a companion study. A total of 84 courses were administered, and the date of last follow-up was March 31, 1998 (median follow-up of 421 days). Patients had documented p53 mutations and histologically determined, viable non-small-cell lung carcinoma as judged by pretreatment tumor biopsies. Patients were treated either percu-

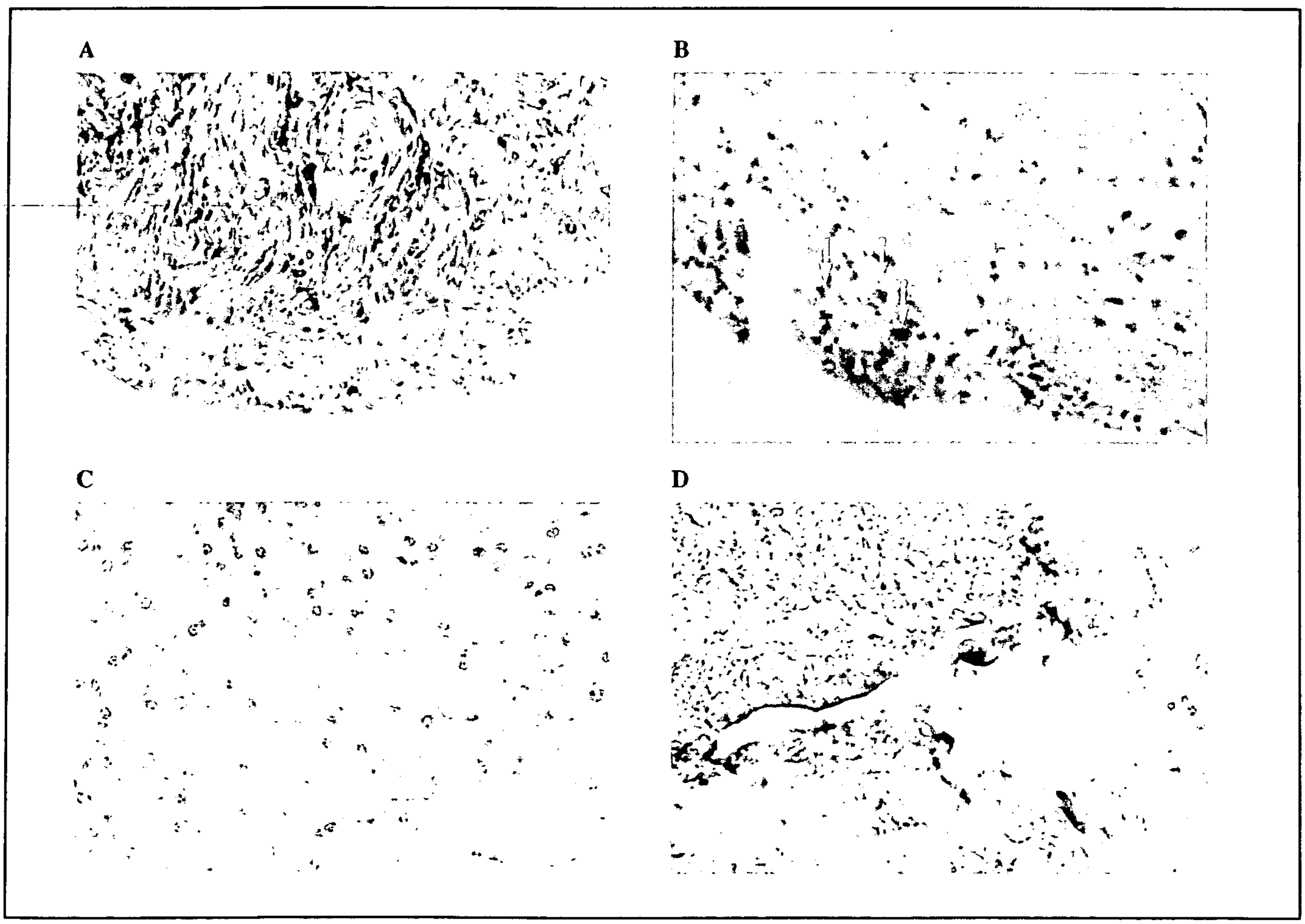

Fig. 1. Staining by terminal deoxynucleotide transferase (TdT)-mediated biotin uridine triphosphate nick-end labeling (TUNEL) technique of left upper lobe tumor biopsy specimens from patient O. Pretreatment (A) and 3 days after injection of 3×10^9 plaque-forming units of Ad-p53 (B). Positive control reactions (C) were performed with deoxyribonuclease-treated sections, while negative control reactions (D) omitted TdT from buffer solution. Arrows outline region of positive nuclear staining, which reflects DNA fragmentation consistent with cells undergoing apoptosis (original magnification ×400).

taneously with the use of CT guidance (23 patients) or bronchoscopically (five patients). Before entry in the study, all patients demonstrated progressing primary or metastatic lesions that had failed to respond to conventional therapies, including surgery in 10 patients (36%), radiation therapy in 25 (89%), and chemotherapy in 21 (75%). Vector-injected tumors were located in the lung parenchyma in 14 patients (50%), chest wall in two patients (7%), bronchus in five patients (18%), liver in five patients (18%), axilla in one patient (4%), and subcarinal node in one patient (4%).

Assessment of Gene Transfer

We performed PCR analysis with vector-specific primers for the adenovirus and p53 sequences to differentiate between vector-transduced p53 and cellular p53 (Table 2). No pretreatment tumor biopsy samples showed evidence of adenoviral p53 DNA (DNA–PCR) or messenger RNA (mRNA) (RT–PCR) sequences. DNA extracted from 18 of 21 evaluable tumors showed vector-specific adenovirus sequences. All patients who received more than 10^6 PFU showed evidence of adenovirus sequences by DNA–PCR in their posttreatment specimens. Vector-specific mRNA p53 sequences were detected by RT–PCR in 12 of 26 evaluable specimens. Transgene expression of p53 was noted in nine of 16 patients treated at doses above 10^9 PFU, as opposed to only three of 10 patients treated with 10^9 PFU or less. Transgene expression of p53 occurred after initial and subsequent treatments at all dose levels above 10^6 PFU.

The mean pretreatment AI was 3.6% (95% CI = 1.5%–5.8%). After administration of Ad-p53, 11 of 24 evaluable patients fell outside the pretreatment 95% CIs with apoptotic indices of 7%–87% in posttreatment tumor biopsy samples (Table 2). No consistent change in inflammatory cell infiltration was seen after Ad-p53 treatment in posttreatment tumor biopsy samples (data not shown).

Adverse Events

Vector-related adverse events were minimal (Table 3). No grade 4 toxicity was seen and grade 3 vector-related toxicity was limited to one incident of nausea after Ad-p53 injection. CT-guided administration of vector resulted in six pneumothoraces that were treated with percutaneous placement of a pigtail catheter in two patients and observation in four patients. Injection site pain was noted during 13 courses (15.5%) and was resolved with oral pain medications in all patients. Four incidents of

Table 2. Assessment of gene transfer and expression in biopsy samples of non-small-cell lung cancer following treatment with Ad-p53, an adenovirus vector carrying wild-type p53 complementary DNA

Patient*	Viral dose, plaque-forming units	No. of courses	DNA–PCR†	RT–PCR†	TUNEL, % positive cells‡	Response§
A	10^6	4	–	–	4	Stable
B	10^6	2	–	–	7‖	Stable
C	10^6	1	–	–	NT¶	Progression
D	10^7	3	+ (2)	NT¶	NT¶	Progression
E	10^7	2	+ (2)	+ (2)	2	Progression
F	10^7	1	+ (1)	+ (1)	7‖	Not evaluable#
G	10^8	3	+ (3)	+ (3)	8‖	Stable
H	10^8	4	+ (1, 2)	–	50‖	Stable
I	10^8	2	+ (1, 2)	–	10‖	Stable
J	10^9	2	+ (1, 2)	–	NT¶	Progression
K	10^9	6	+ (1, 3, 5, 6)	–	4	Partial response
L	10^9	3	+ (3)	–	4	Stable
M	3×10^9	4	+ (3, 4)	+ (4)	12‖	Stable
N	3×10^9	2	+ (1, 2)	+ (1)	4	Stable
O	3×10^9	6	NT¶	+ (1, 2, 6)	46‖	Partial response
P	3×10^9	2	NT¶	–	3	Stable
Q	10^{10}	5	+ (2, 4, 5)	–	50‖	Stable
R	10^{10}	2	+ (1, 2)	+ (1, 2)	NT¶	Stable
S	10^{10}	5	+ (1, 2, 3, 4)	–	7‖	Stable
T	3×10^{10}	2	NT¶	+ (1)	4	Progression
U	3×10^{10}	1	NT¶	–	0	Not evaluable#
V	3×10^{10}	6	+ (1, 2, 3)	–	87‖	Stable
W	10^{11}	3	NT¶	+ (2, 3)	7‖	Progression
X	10^{11}	6	+ (1, 3, 4, 5, 6)	–	0	Stable
Y	10^{11}	1	NT¶	+ (1)	2	Not evaluable#
Z	10^{11}	2	NT¶	–	3	Stable
AA	10^{11}	3	+ (2, 3)	+ (2)	1	Stable
BB	10^{11}	1	+ (1)	+ (1)	1	Progression

*Sequential letters were assigned during manuscript editing to ensure confidentiality of patients; the letters do not represent identifiers used during the trial.

†Course number during which DNA–polymerase chain reaction (PCR) or reverse transcription (RT)–PCR was positive is given in parentheses.

‡Maximum percentage of cells staining positive by terminal deoxynucleotide transferase-mediated biotin uridine triphosphate nick-end labeling (TUNEL) posttreatment. Mean pretreatment apotolic index was 3.6% with 95% confidence interval = 1.5%–5.8%.

§Partial response = decrease ≥50% in size of tumor for minimum of 4 weeks; complete response = complete disappearance of tumor as judged by computed tomography scan and physical examination for a minimum of 4 weeks; progression = increase ≥25% in size of tumor; stable = any variation in size not meeting criteria of complete response, partial response, or progression.

‖Posttreatment apoptotic index above 95% confidence interval of pretreatment apoptotic index.

¶NT = not tested because of insufficient quantity or quality of biopsy specimen.

#Not evaluable because patients died of non-treatment-related causes prior to 30-day follow-up computed tomography scan.

Table 3. Adverse events associated with Ad-p53 (adenovirus vector) gene therapy in patients with non-small-cell lung cancer*

Adverse event	No. of courses†	Grade 1‡	Grade 2‡	Grade 3‡	Grade 4‡	Total§
Fever	84	13 (15.5)	10 (11.9)	0	0	23 (27.4)
Injection site pain	84	6 (7.1)	6 (7.1)	1 (1.2)	0	13 (15.5)
Pneumothorax	84	3 (3.6)	2 (2.4)	1 (1.2)	0	6 (7.1)
Nausea	84	3 (3.6)	0	1 (1.2)	0	4 (4.8)
Hemoptysis	84	2 (2.4)	2 (2.4)	0	0	4 (4.8)
Chills	84	1 (1.2)	1 (1.2)	0	0	2 (2.4)
Anorexia	84	1 (1.2)	0	0	0	1 (1.2)

*Toxicity defined by National Cancer Institute Common Toxicity Criteria (grades 1–4).

†Total number of courses of Ad-p53 administered during the trial.

‡Highest grade toxicity associated with Ad-p53 treatment. Percentage of courses with this level of toxicity is shown in parentheses.

§Total number of each adverse event (grades 1–4) associated with Ad-p53. Percentage of courses with the adverse event is shown in parentheses.

transient hemoptysis were noted after bronchoscopic injection and were resolved with observation. The most common vector-associated adverse event was fever, occurring 6–24 hours after injection in 23 treatments (27.4%). These fevers were treated with antipyretics or observation and resolved within the next 24–48 hours. There was no increase in adverse events with repeat treatments or higher doses of Ad-p53, and dose-limiting Ad-p53 toxicity was not reached in this trial. In addition, no patient demonstrated hypotension or anaphylaxis despite repeated (up to six) doses of Ad-p53.

Effect on Tumor Growth

Clinical response of the injected tumor was evaluable in 25 patients (89%) and included the following: partial response in two patients (8%; 95% CI = 1%–26%), stabilization of disease in 16 patients (64%; 95% CI = 43%–82%) (range, 2–14 months), and progression of disease in seven patients (28%; 95% CI = 12%–49%). Three patients were not evaluable because they died from treatment problems unrelated to Ad-p53 before a 30-day follow-up CT scan was done. Of note, three of five patients who received less than 10^8 PFU of Ad-p53 showed continued progression of their disease while on treatment, whereas only four of 22 patients receiving 10^8 PFU or more showed disease progression. There was no clear relationship between patient characteristics, adverse events, gene expression, or tumor location/size/histology and clinical response. Details of the two patients (patients K and O) who demonstrated a partial response following Ad-p53 gene therapy are as follows:

Patient K presented with a left upper lobe large-cell carcinoma on November 1994. Because of poor results on pulmonary function tests, the patient was judged not to be a surgical candidate and was treated with 66 Gy of external beam radiation therapy. The primary tumor recurred in February 1996 and was treated with six cycles of paclitaxel and carboplatin. The patient was subsequently enrolled in the gene therapy protocol because the tumor had progressed. At a dose level of 10^9 PFU of Ad-p53, the tumor responded with a greater than 50% decrease in size (Fig. 2). No viable tumor was demonstrated on tumor biopsies after the first two courses of Ad-p53 therapy. After completion of gene therapy in June 1997, the patient was observed without further treatment and, at the time of the last follow-up (March 1998), showed no evidence of recurrent disease.

In September 1994, patient O was found by bronchoscopy to have an adenocarcinoma that was partially obstructing the left upper lobe. The patient was treated with two courses of cisplatin and etoposide, followed by 60 Gy of definitive external beam radiation therapy. In December 1995, the tumor recurred with bronchial obstruction of the left upper lobe and was treated with laser therapy and 21 cycles of mitomycin C and navelbine. One year later, in December 1996, the patient's left upper lobe of the bronchus was found to be reoccluded; laser therapy was attempted but failed. Direct intratumoral injection of 3×10^9 PFU of Ad-p53 was begun in December 1996, resulting in a partial response and reopening of the airway (Fig. 3). This response was maintained for 6 months with Ad-p53 alone. At the completion of therapy, residual tumor still remained and three additional courses of carboplatin and docetaxol were given, resulting in a complete histologic response. One year later, in December 1997, the tumor recurred, and the patient was begun on a follow-up Ad-p53 protocol, which was discontinued because of further tumor progression.

The median survival of all patients from the time of initiating gene therapy was 141 days (Fig. 4). At the time of last follow-up, five patients were still alive greater than 10 months after initiating therapy and two patients were being observed off all treatment without evidence of tumor growth.

Discussion

The estimated incidence of lung cancer in the United States in 1997 was 178 100, with more than 160 400 deaths *(29)*. Despite advances in chemotherapy, radiation therapy, and surgery, overall survival for this disease is still less than 13% *(29)*. Because of the poor results obtained with conventional therapy alone, additional treatment strategies are needed. Our study evaluated the novel strategy of intratumoral injection of an adenovirus vector expressing wt-p53 (Ad-p53) in patients with advanced NSCLC whose cancers had failed to respond to conventional treatments.

One important finding of this study was that multiple doses of Ad-p53 could be administered safely. We have reported previously *(28)*, in an article that described findings for 12 of the patients in this study, that neutralizing anti-adenovirus antibodies rise sharply in the serum after the first course of Ad-p53 and remain elevated throughout therapy. Despite this fact, we observed no major vector-related toxicity with repeat injections. A total of 84 doses of Ad-p53 were delivered; 56 of these doses were repeat injections (up to six injections given monthly). There were no anaphylactic reactions or episodes of hypotension and only one grade 3 vector-related adverse event (nausea) during treatment. All other toxic effects were grade 1 or 2 and consisted primarily of transient fevers treated with antipyretics. Repeated delivery of the vector by CT guidance or bronchoscopy was also feasible, and only six pneumothoraces developed during 84 injections. These pneumothoraces were treated with observation in four patients and with placement of a pigtail catheter in two patients. In addition, pain at the site of injection was noted in only 13 of 84 courses of therapy. Such pain was treated with analgesics and resolved within the first 24–48 hours of injection in most cases. Clayman et al. *(30)* also noted pain at the injection site as being the most common adverse event after intratumoral injection of Ad-p53 in patients with recurrent head and neck squamous cell carcinoma. In our study, a maximum tolerated dose was not reached. Dose escalation was limited by

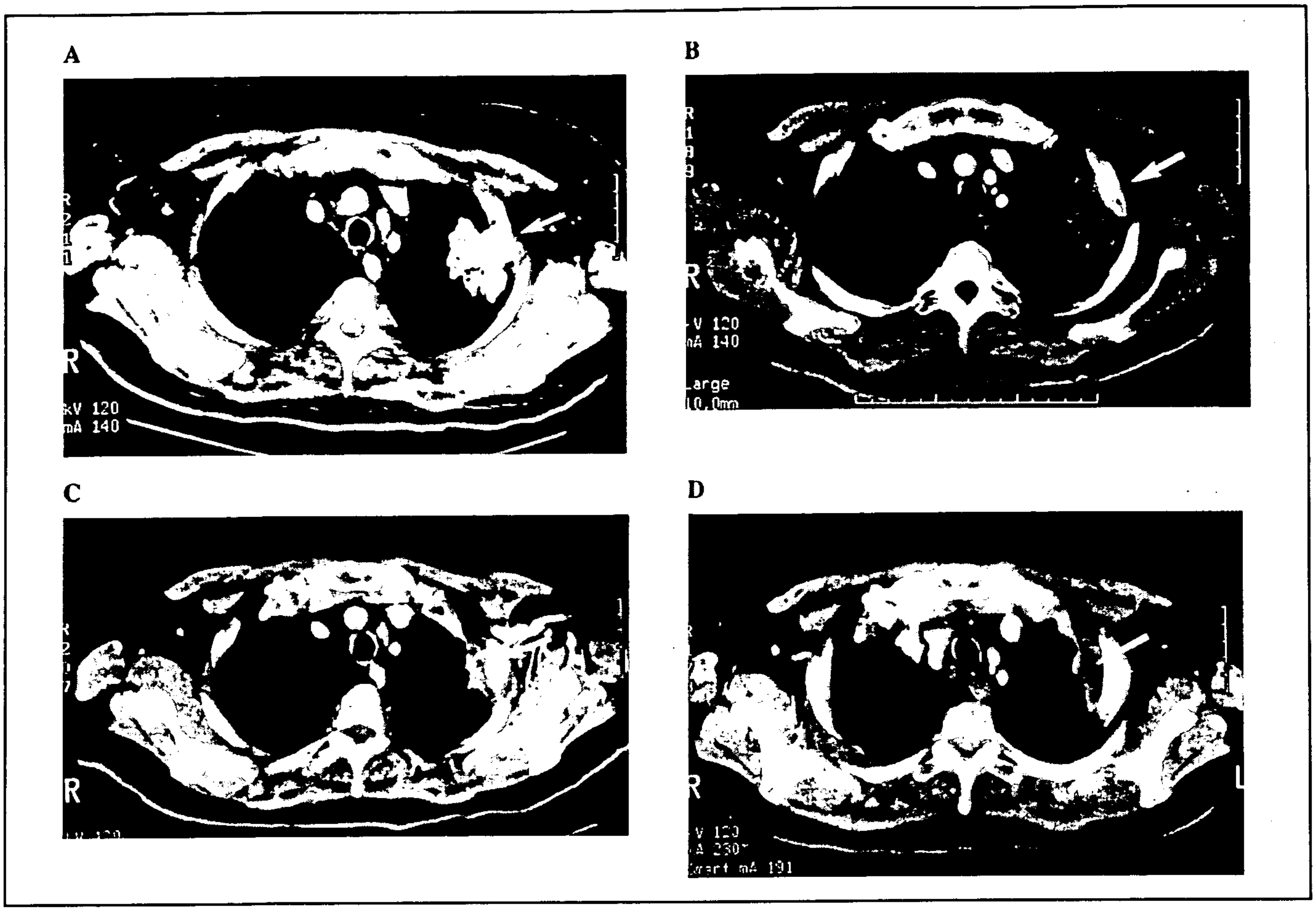

Fig. 2. Computed tomography (CT) scans of patient K following six courses of 10^9 plaque-forming units of Ad-p53, an adenovirus vector carrying the wild-type p53 complementary DNA. **A)** Before treatment, **Arrow** shows recurrent left upper lobe adenocarcinoma, which progressed after 66 Gy of external beam radiation therapy and six courses of paclitaxel and carboplatin (CT scan volume: $3 \times 4 \times 5$ cm = 60 cm^3). **B)** At 1 month after treatment, **arrow** shows tumor regression after one course of Ad-p53 treatment (CT scan volume: $2 \times 3 \times 5$ cm = 30 cm^3). **C)** At 8 months after treatment, image shows tumor regression following six courses of Ad-p53 gene therapy (CT scan volume: $2 \times 2 \times 3$ cm = 12 cm^3). **D)** Stable tumor 18 months after beginning treatment with Ad-p53 (CT scan volume: $2 \times 2 \times 3$ cm = 12 cm^3). No viable tumor was demonstrated during the last 4 months of therapy (14 sequential percutaneous biopsies), and the patient was observed off all treatment for 12 months without evidence of tumor progression.

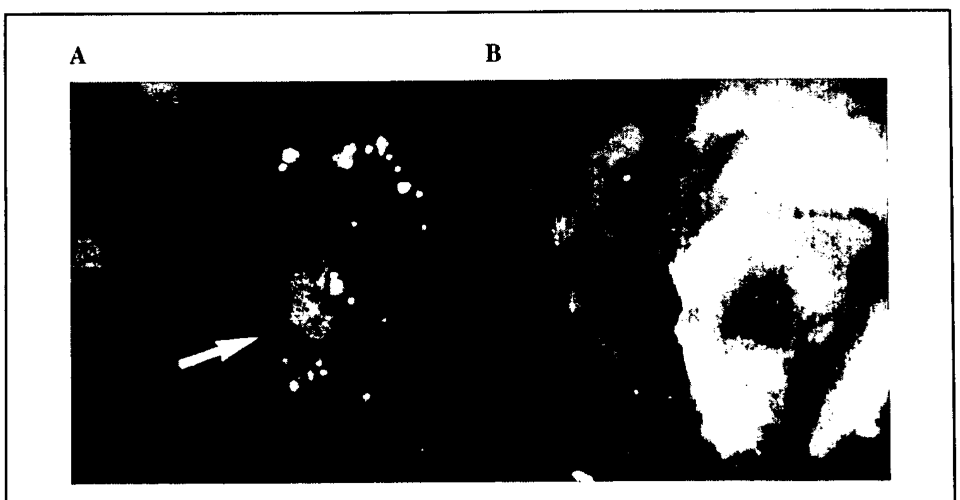

Fig. 3. Bronchoscopic images of patient O before treatment (**A**) and 30 days after treatment with 3×10^9 plaque-forming units of Ad-p53, an adenovirus vector that carried the complementary DNA encoding wild-type p53 protein (**B**). **A)** The tumor is obstructing the left upper lobe. **B)** Patient's left upper lobe airway 30 days after treatment. The response was maintained for 6 months with Ad-p53 alone.

the protocol to 10^{11} PFU. Similarly, Sterman et al. *(31)* found that a dose-limiting toxicity was not reached after intrapleural instillation of up to 10^{12} PFU of an adenovirus vector, Ad-HSV-tk, containing the herpes simplex virus–thymidine kinase gene into patients with mesothelioma. Because of the low toxicity, 10 patients at the end of the study were able to be treated as outpatients rather than as inpatients, even though six of them were receiving the highest dose of Ad-p53. This low toxicity is im-

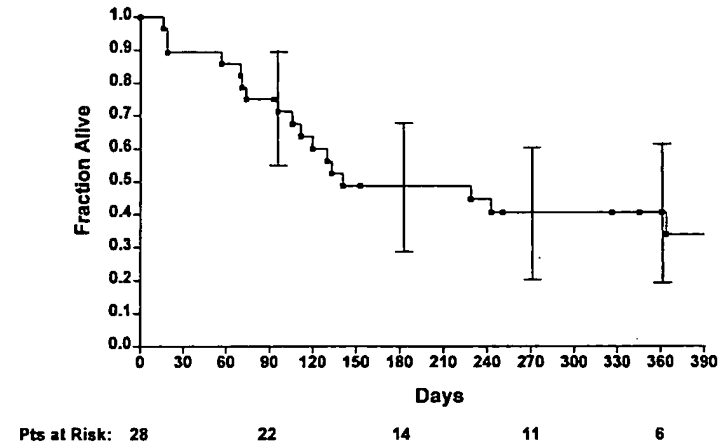

Fig. 4. Actuarial survival of all patients treated with Ad-p53, an adenovirus vector carrying the complementary DNA sequence encoding wild-type p53. The Kaplan–Meier method was used to assess survival (95% confidence intervals = error bars). Pts = patients.

portant for future trials because it means that Ad-p53 has potentially a high therapeutic index that allows it to be used in combination with other conventional treatments, such as chemotherapy, radiation therapy, or surgery.

The second important observation of this trial was that wt-p53 cDNA transfer and expression could be accomplished and documented in a large number of patients. We used vector-specific primers that incorporated flanking regions of the adenovirus to ensure detection of adenovirally transferred p53 mRNA rather than of native p53. Adenovirus-mediated p53 cDNA transfer appeared to be dose related. Transgene p53 expression could be documented by RT–PCR in 56% of the patients treated with 10^9 PFU or more, whereas only 30% of patients who received lower doses showed transgene p53 expression (Table 2). Importantly, Ad-p53 transgene expression could still be observed after multiple courses even in the presence of high serum levels of anti-adenovirus antibodies. Sterman et al. *(31)* also noted dose-related gene expression when mesothelioma patients were treated with intrapleural instillation of Ad-HSV–tk. In addition, Tursz et al. *(32)* documented increasing β-galactosidase expression in patients with endobronchial lung cancer following intratumoral injection of higher doses of an adenovirus vector containing the β-galactosidase gene. Since maximal adenovirus expression occurs *in vitro* at 3 days and drops off rapidly during the next week, our observation that transgene expression still occurs with multiple courses may be important for future clinical trials that require prolonged periods of transgene expression. Detection of gene expression following transfer of wt-p53 *in vivo* is difficult because successful transfer and expression of wt-p53 in a tumor may compromise evidence of gene expression by the rapid induction of apoptosis. Other constraints on detection of p53 transgene expression in this study are the small size and scant cellularity of the biopsy specimens and the low sensitivity of the RT–PCR assay *(33)*. These findings may explain in part the variation noted in PCR and RT–PCR assays performed on sequential biopsy samples.

The third important finding was that evidence of antitumor activity was suggested following Ad-p53 cDNA transfer. Two patients demonstrated a greater than 50% reduction in tumor size after Ad-p53 injection. In one patient, no viable tumor cells could be demonstrated in all biopsy specimens obtained during the last 4 months of treatment. Because of a lack of histologic evidence of cancer following treatment with Ad-p53 alone, we elected to observe this patient off all therapy; at last follow-up—more than 18 months after starting treatment—the patient was without evidence of recurrent tumor. Patients with endobronchial tumors may also represent a subset of patients who could benefit from Ad-p53 treatment, since we observed almost complete regression of a left upper lobe endobronchial tumor that had failed to respond to chemotherapy, radiation therapy, and laser treatment. To our knowledge, this is the first study to demonstrate sustained antitumor activity in NSCLC with gene transfer of wt-p53 alone. Since these patients had already failed to respond to multiple other treatments, future trials with untreated patients or with patients with earlier stage disease may have higher response rates. In addition, Clayman et al. *(30)* noted that Ad-p53 resulted in the partial regression of disease in two of 17 evaluable patients with recurrent head and neck carcinoma. These results suggest that the antitumor activity noted with Ad-p53 treatment may be effective in other types of cancer. Because of heterogeneity in patients and tumors entered in this phase I study, definitive statements about clinical efficacy are difficult. No clear association could be demonstrated between patient and tumor characteristics and response; however, it did appear that higher doses of Ad-p53 ($>10^8$ PFU) were associated with longer times to disease progression. It is interesting that both patients who responded demonstrated continued disease regression with repeated administrations of Ad-p53, even in the face of elevated anti-adenovirus antibodies. Li et al. *(34)* have shown in an immunocompetent mouse model that multiple intratumoral injections of Ad-p53 result in increased tumor regression and transgene expression despite elevated levels of circulating adenovirus antibodies. These observations validate the strategy of administering multiple intratumoral injections of Ad-p53 to maximize transgene expression and tumor response.

Although immune-mediated responses have been reported after adenovirus treatment *(35)*, we observed no evidence of a substantial increase in inflammatory cell infiltrates in posttreatment tumor biopsy specimens. In addition, despite increases in neutralizing levels of anti-adenovirus antibodies and increased lymphocyte proliferative responses to adenovirus serotype 5 antigens, no antibody-dependent cytotoxicity could be demonstrated in posttreatment serum samples, and changes in lymphocyte proliferative responses to p53 mutant and wild type were not observed (Yen N, Ioannides CG, Xu K, Swisher SG, Lawrence DD, Kemp BL, et al.: unpublished observations).

Moreover, preclinical studies have demonstrated in both immunocompetent and immunodeficient animal models an antitumor activity that appears to be p53 specific *(7,8,10,12)*. It is unlikely, therefore, that the antitumor activity we observed in this trial was due to immune-mediated effects. Another possible mechanism for the antitumor effects is the induction of apoptosis by Ad-p53. We have observed that the transduction of human lung cancer cells with Ad-p53 results in the increase of the proapoptotic Bcl-2 family members Bax and Bak *(36)*. This mechanism is supported by the high levels of apoptosis seen with TUNEL staining in the posttreatment tumor biopsy samples from 11 of 24 evaluable patients. In the two patients who responded to Ad-p53, one patient demonstrated a posttreatment AI of 46%, while the other patient could not be evaluated because of the large amount of necrosis after treatment. In addition, since posttreatment tumor biopsies were always performed 3 days after Ad-p53 injection, the critical period of apoptosis may have been missed in some patients because *in vitro* studies *(37)* suggest that apoptosis can occur as soon as 3 hours after induction. It is interesting, however, that, in those patients who demonstrated TUNEL activity, TdT expression was markedly higher than that reported in patients following chemotherapy. Ueda et al. *(38)* noted only two of 22 patients with an AI greater than 10% after intra-arterial infusion of chemotherapy in cervical carcinoma, whereas we noted four patients with an AI ranging from 47% to 87% after Ad-p53 treatment (Table 2). Intratumoral injection of retroviral p53 has also been associated with increased TUNEL activity *(14)*, which suggests that induction of apoptosis by transgene expression of wt-p53 may be one of the mechanisms underlying tumor regression.

In summary, this study demonstrates for the first time, to our knowledge, the clinical feasibility of adenoviral p53 cDNA transfer strategies in NSCLC. The safety and efficacy of repeated doses, even in large, established tumors, suggest that a therapeutic window exists during which clinical benefit is not accompanied by additional toxicity. Several potential clinical applications of this technology exist. At the present time, local control of lung cancer remains suboptimal. Radiation therapy is limited in effectiveness because of toxicity to normal tissues at higher doses. Since preclinical studies suggest synergism between radiation and Ad-p53 without increased toxicity, one potential strategy might be to combine the two modalities for enhanced local tumor control *(39)*. Another possibility is in premalignant lesions, such as bronchial dysplasias, where p53 mutations are known to precede invasive carcinoma *(40,41)*. Localized injection of a nontoxic agent such as Ad-p53 might ultimately play a role in preventing the development of invasive cancer. Phase II clinical trials are now under way to determine the feasibility of these strategies and to determine the clinical role of Ad-p53 in the treatment of lung cancer.

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Notes

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:n shown to be decr ased in OPLs and these othesis that loss of RARβ is associated with opment. A chemoprevention study with OPL ng/Kg/d) for 3 months has demonstrated an ciation with clinical response. The purpose of ine levels of RARβ and the upregulation of its :reatment at the above dose of 13CRA or after lower 13CRA dose (0.5 mg/Kg/d) to cancer receptor level data that were available for 53 atients at 3 months, and for 35 patients at 12 3 of 18 patients who developed cancer after a d that lack of RARβ expression in OPLs does ient, and upregulation of RARβ in patients 12 months does not predict a lower cancer ie time to development of cancer was not e conclude that RARβ expression does not opment, and its loss does not appear to be icer incidence. It is possible that progression occurred after discontinuation of the 13CRA sociated with loss of response to putative ession and eventual cancer development in a irted by USPHS Grant NCI CA46303 from the

F METHOTREXATE (MTX) VERSUS TWO SCHED-'N PATIENTS WITH RECURRENT OR METASTATIC OF THE HEAD AND NECK (SCCHN). J.B. Ver-oekman, P.H.M. de Mulder, J. Wildiers, the EORTC Head & Neck Cancer Cooperative

:s have indicated that Taxol (T) has substan-with advanced SCCHN. Its real value in a ially investigated in a direct comparison with fore, we evaluate the antitumor activity and ion schedules of T with weekly MTX in an hase II study. T is given at a dose of 175 sion or a 24-h infusion, while MTX is given at jection. Dose escalation is carried out in all 3 growth factors are not allowed. Eligibility :rapy for recurrence, age 18–75 years, WHO measurable or evaluable disease, adequate sent. Pts are stratified by institution, PS, To date (Dec. 1997) 124 pts have been their treatment and 76 so far could be

(n = 28)	3-h T (n = 27)	24-h T (n = 27)
29	0	22
4	7	52
4	4	41
14	0	7

alopecia, mild myalgia and (mainly mild) esponses have been observed in the 76 ie by arm will be presented in May 1998.

antitum r activity; the 24-h T schedule s adverse events.

This study was conducted on 82 advanc d head and neck cancer patients (71 men, 11 women; mean age 59; 2 T1, 6 T2, 38 T3, 35 T4). Induction treatment was cisplatin-FU ± folinic acid chemotherapy for 61 patients and concomitant chemo (cisplatin-FU)—radiotherapy for 21 patients. EGFR (binding assay), p53 protein (immunoluminometric assay, Sangtec) and TS activity (radioenzymatic assay) were measured on tumoral biopsies obtained at time of diagnosis. EGFR ranged up to 3930 fmol/mg (mean 356, median 110), p53 up to 24.56 ng/mg (mean 1.80, median 0.19) and TS up to 81780 fmol/mn/mg (mean 4320, m dian 1660). A significant positive correlation was demonstrated between p53 and EGFR. No relationship was observed between p53 and TS activity. Tumoral response was: 39 CR, 25 PR, 17 failures. N ither EGFR, p53, nor TS were significantly different between CR and PR + failure. In the chemo-radiotherapy group, p53 was higher in non-responders (median 1.03) than in complete responders (median 0.08) (Mann-Whitney, $p = 0.057$). Thirty-three patients died from their cancer. Univariate Cox analyses showed that tumor size (T1–2–3 vs T4), EGFR status (below vs over 220) and p53 status (below vs over 0.80) were significant predictors of specific survival ($p < 0.002$), survival being increased when EGFR and p53 were below thresholds. A multivariate stepwise analysis including tumor size, EGFR and p53 revealed that tumor size and EGFR were the only independent predictors of survival ($p < 0.002$); relative risks were 4.36 for tumor size and 3.06 for EGFR. The independent predictive values of tumor size and EGFR remained significant after stratification on treatment type. When response to treatment was entered in a stepwise analysis including tumor size, EGFR and p53 status, the remaining prognostic factors were tumor size ($p = 0.0006$), EGFR ($p = 0.004$) and treatment response ($p - 0.02$). Such data may be helpful to improve treatment management of advanced head and neck cancers.

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A PHASE II TRIAL OF INTRATUMORAL INJECTION WITH AN E1B-DELETED ADENOVIRUS, ONYX-015, IN PATIENTS WITH RECURRENT, REFRACTORY HEAD AND NECK CANCER. D. Kirn, J. Nemunaitis, I. Ganly, M. Posner, E. Vokes, J. Kuhn, C. Heise, C. Maack, S. Kaye. *Physicians Reliance Network, Dallas, TX; Dept. of Medical Oncology, Beatson Oncology Center, Glasgow Scotland; Dana Farber Cancer Institute, Boston, M.A.; Dept. of Medical Oncology, University of Chicago, Chicago, IL; Onyx Pharmaceuticals, Richmond, CA.*

ONYX-015 is an E1B-deleted group C adenovirus which selectively replicates in and lyses tumor cells that are deficient in p53 function (*Nature Medicine 1997; 3:639*). ONYX-015 was injected intratumorally daily for 5 consecutive days (10^{10} plaque-forming units per day) in patients with recurrent squamous cell carcinoma of the head and neck. All patients had unresectable tumors that had progressed on chemotherapy and/or radiation following tumor recurrence. p53 sequencing is being performed on all tumors. Treatment cycles were repeated every 3 weeks. Primary endpoints include the target (injected) tumor response rate, time to target tumor progression, pain response (visual analogue scale), neutralizing antibody response and safety. Twenty-one patients have been treated to date. Grade 1–2 flu-like symptoms were seen in approximately 90% of patients. Two patients experienced grade III tumor injection site pain requiring premedication with analgesics on subsequent courses. Thirteen patients are evaluable for response at this time. Two tumors achieved partial response (>50% regression). Two underwent complete regression. Five patients had stable target tumors lasting 6+ to 12+ weeks. Baseline and post-treatment neutralizing antibody titers to ONYX-015 are pending. Symptomatic improvement was reported in three patients, including improved jaw mobility (n = 2) and improved speech (n − 1). ONYX-015 was well-tolerated and showed antitumoral activity leading to subjective clinical benefit in refractory, recurrent head and neck cancer patients.

Exhibit 11